Journal of Life Sciences & Biomedicine, vol. 4(77), No 1, p. 05-12 (2022)

Stability, plasticity, and adaptability of winter bread wheat under rainfed conditions with unstable moisture supply

Jahangirov Atabay Agasoltan

Gobustan Regional Experimental Station, Research Institute of Crop Husbandry, Ministry of Agriculture of the Republic of Azerbaijan, Gobustan AZ 3700, Azerbaijan
For correspondence: *a.cahangir@hotmail.com*

Received: January 17, 2022; Received in revised form: March 10, 2022; Accepted: March 16, 2022

Productivity, stability, plasticity, and adaptability of 14 varieties and 1 line of winter bread wheat were studied in Mountenous Shirvan, under rainfed conditions with no stable humidıty, in 2010/2011-2017/2018 vegetation years. Based on hydrometeorological indicators, the climatic conditions of 2010-2011, 2012-2013, 2016-2017, and 2017-2018 vegetation years were considered favorable for the development of winter wheat, and the climatic conditions of 2014-2015 and 2015-2016 were moderate and the climatic conditions of 2011-2012 and 2013- 2014 were found to be unfavorable. In 2011, 2013, 2017, and 2018, the average productivity for all genotypes was the highest and amounted to 61.3, 69.8, 66.9, and 66.6 cwt/ha, respectively. In 2015 and 2016, productivity was moderate, while in 2012 and 2014, it was at a low level. The average productivity over the years was in line with the climatic conditions during the vegetation year. According to the average of 8 years, Gobustan, 7th WON-SA №465, and Gyrmyzy gul 1 genotypes had the highest productivity - 66.4, 63.4, and 60.4 cwt/ha, respectively. Azeri, Bezostaya 1, Murov 2, Ruzi 84, and Sheki 1 varieties showed the lowest results. Taking into account the stability and plasticity of the studied varieties, it was concluded that Bezostaya 1, Ruzi 84, and Sonmez 01 varieties belong to the neutral type with the least plasticity (i.e. the type with wide adaptability). The varieties 7 thWON-SA №465 and Gobustan manifested a specific adaptation with high plasticity, increased productivity in response to high agrophone and favorable climatic conditions, as well as adaptability to adverse conditions. Therefore, the cultivation of these varieties in areas with unstable moisture supply, such as Mountainous Shirvan, and in a high agrophone, creates conditions for obtaining good results. In addition to not being adapted to unfavorable conditions, Tale 38 and Azamatli 95 varieties were found to have narrow adaptability and high plasticity in response to improved conditions.

Keywords: *Winter bread wheat, rainfed conditions, productivity, stability, plasticity, adaptability*

INTRODUCTION

Increasing wheat production and meeting the demand for cereals at the expense of local production is an important aspect of food security. Achieving the predicted level is possible with the use of high-yielding local varieties of cereals. This, in turn, should include highly adaptable and potentially productive varieties that are tolerant to biotic and abiotic stress factors at the level of agrocenosis, agroecosystems, and agroecological landscapes (Zhuchenko, 2001; Aliyev, 2012; Aliyev, Huseynova, 2014). Autumn cereals dominate over spring cereals due to yield potential and early ripeness. They better use natural soil-climate resources, including spring warmness and moisture. Besides, they are less subjected to the spring drought effect. Therefore, autumn cereals are very important for total grain production. The introduction of varieties with high adaptive potential into production is one of the conditions that allow production to be stabilized even in years that differ in humidity and temperature (Samofalova et al., 2019). In this case, the varieties must be ecologically plastic and have an individual reaction to changes in climate and meteorological conditions.

10.5281/zenodo.7197120

The study of the reaction to changes in the cultivation conditions of the variety can allow the selection of a variety that meets the needs of agriculture for each ecological region and farm (Al- iev, 2000, 2001; Mameev, Nikiforov, 2015; Pe- trov, Selekhov, 2016). Productivity is an im- portant indicator for assessing the ecological plas-ticity and stability of a variety, as it determinesthe reaction to the improvement of growing con- ditions and also indicates the level of intensity of cultivation technology (Sharma et al., 2012,2013). The adaptability of a variety to environ- mental conditions is judged by its environmental plasticity and yield stability. Plasticity is an adap- tive reaction of genotypes to the effects of environmental factors that cause changes in productivity and other traits of the organism. The term "average productivity of varieties" (P-average) is used to define productive and adaptive potential using a variation of the productivity under a spe- cific condition or region. In this case, the produc- tivity of the variety is compared with the "averageyield of varieties" rather than the standards. To assess the response of any variety to the condi- tions of the vegetation period, its productivity is divided by the average yield, and the value ob- tained indicates the coefficient of adaptability as arelative concept (Mameev, Nikiforov, 2015). Sta- bility is the degree of resilience that characterizesa genotypic adaptive reaction leading to changesin signs and characteristics of the organism against changing environmental factors. Varieties that have moderate intensity and high yield stabil-ity but are not highly productive under any condi- tions are considered ecologically tolerant ones.

It is known that the climatic conditions in the Mountainous Shirvan region changed over the years, which had a serious impact on production. Thus, although favorable agrometeorological conditions lead to an increase in crop production, in some years, drought caused by low precipitation, and sometimes accompanied by high temperatures, leads to serious crop losses. In this case, the selection and application of adaptive and relatively plastic varieties in the region would not only create conditions for a significant increase in production in favorable years but also ensure stable production during unfavorable years. From this point of view, the study of productivity, yield stability, plasticity, and adaptability of new varieties under rainfed conditions of Mountenous Shirvan with unstable moisture supply is of great importance.

MATERIALS AND METHODS

The studies were performed on 15 wheat genotypes with contrasting morphophysiological traits and productivity, under the conditions of unstable moisture supply of Mountainous Shirvan in the vegetation years of 2010-2011, 2011-2012, 2012-2013, 2013-2014, 2014-2015, 2015-2016, 2016-2017, and 2017-2018. The experiments were conducted on arable lands of the Gobustan Regional Experimental Station of the Research Institute of Crop Husbandry located in foothills. The area is at an altitude of 800 m above sea level. The soil type is light chestnut and has a weak alkaline environment. The region has rainfed conditions with unstable moisture supply and according to the average multi-year data, the amount of atmospheric precipitation is 350.0-400.0 mm.

The studied genotypes were planted in 3 repetitions in the form of randomly placed blocks with an area of 32.0 m^2 in each experimental unit, the sowing norm was 450 seedlings per 1 m^2.In the full ripeness phase, the experimental units were mowed with a mini-experimental combine "Foton Gushen" and the product was weighed. The results were analyzed using "JMP 5.0.1" and "Genstat" programs.

RESULTS AND DISCUSSION

During the 2010/2011-2017/2018 vegetation years (8 years in total), 14 varieties and 1 line of winter wheat were studied to assess productivity, yield stability, plasticity, and adaptability of new varieties under rainfed conditions of Mountenous Shirvan with unstable moisture supply. Information on climatic conditions from 2010 to 2018, when the research was conducted, was obtained from the Gobustan hydrometeorological station. Based on the above indicators, the climatic conditions of 2010-2011, 2012-2013, 2016-2017, and 2017-2018 vegetation years were considered favorable for the development of winter wheat, and the climatic conditions of 2014-2015 and 2015-

2016 were moderate and the climatic conditions of 2011-2012 and 2013- 2014 were found to be unfavorable. The results of the study of the productivity of varieties during the research years are given in Table 1. As seen in the table, differences in a significance level of 0.01 were observed between the studied genotypes. For each year, the least significant difference (LSD) between the genotypes was determined, and based on this value, the genotypes were assigned to different groups. At the same time, the average productivity for the years is given in Table1. As seen in the table, the average productivity in 2011, 2013, 2017, and 2018 was at the highest level and amounted to 61.3 (C), 69.8 (A), 66.9 (B), and 66.6 (B) cwt/ha, respectively (based on LSD, a designation of the group related to a year is given in the parenthesis). Productivity was at a moderate level in 2015 and 2016, and a low level in 2012 and 2014. As can be seen, the average productivity over the years was consistent with the course of climatic conditions during the vegetation season.

The results of the average productivity of the studied varieties for all years are given in Figure 1. As seen in the figure, the average productivity for all genotypes was 55.1 cwt/ha. According to the 8-year average values, Gobustan, 7thWON-SA №465, and Gyrmyzy gul 1 varieties showed the highest results - 66.4 (A), 63.4 (B), and 60.4 (C) cwt/ha, respectively. Azeri (H), Bezostaya 1 (H), Murov 2 (H), Ruzi 84, (G), and Sheki 1 (G) varieties showed low productivity, while other studied genotypes were in the middle position (Based on LSD, a group designation of the variety is given in parentheses).

To study yield stability, plasticity, and adaptability of the varieties, AMMI (“Additive main effects of anova along with the multiplicative interaction” effects of Principal Components Analysis) analysis was performed using the “Genstat” program. As a result of the AMMI analysis, a linear dependence of the productivity of varieties on the environmental factor was established, and on the basis of these dependencies, linear regression equations in the form of the formula y = a + bx were obtained for each variety. These equations are given below:

7thWON-SA №465: productivity (cwt/ha)=1.56+1.12*Environmental index

ARAN: productivity (cwt/ha)=2.72+0.92* Environmental index

Azeri: productivity (cwt/ha)=-0.90+0.90*Environmental index

Table 1. Productivity of t ıe studied genotypes by research years

Genotypes	Productivity, cwt/ha								
	2011	2012	2013	2014	2015	2016	2017	2018	Average
7thWON-SA №465	76.2 b	30.4 abc	79.8 ab	55 a	57.8 b	60.3 ab	74.6 ab	73.1 b	63.4 B
Aran	63.4 e	27.7 bcde	62.8 f	45.9 de	53.2 de	45.3 fg	69.6 cde	60.8 efg	53.6 EF
Azeri	47 h	25.5 de	66.8 de	44.2 efg	48.8 f	37.1 ı	59.6 h	60.4 fg	48.7 H
Bezostaya 1	41.1ı	25.6 de	62.1f	44.6 defg	49.1 f	39.6 hı	61.3 h	63.1 def	48.3 H
Azamatli 95	48.2 h	20.1 f	67.3 de	43.7 efg	57.6 bc	54.2 c	68.3 def	75.7 b	54.4 E
Guneshli	69.7 cd	30.5 abc	74 c	51.1 abc	55.2 bcd	53.4 c	67.9 defg	69.6 c	58.9 D
Murov 2	56.6 g	24.5 e	70 d	41.3 g	43 g	38.3 hı	59.6 h	59.2 g	49.1 H
Gyrmyzy gul 1	73.8 bc	31.5 ab	76.7 bc	53.9 ab	56.8 bcd	53.5 c	67.5 defg	69.6 c	60.4 C
Gyzyl bughda	65.5 de	27.5 cde	63.3 f	45 defg	49.6 ef	44 g	70.4 cd	64.6 d	53.7 EF
Gobustan	82.5 a	31.7 a	81.8 a	54.6 a	63.4 a	62.1 a	75 a	80.4 a	66.4 A
Ruzi 84	50.6 h	26.2 de	70.1 d	45.4 def	48.5 f	51.7 cd	60.5 h	59.4 g	51.6 G
Sonmez 01	57.7 fg	28.8 abcd	62.7f	50.2 bc	55.7 bcd	47.4 ef	66.8 efg	64.3 d	54.2 EF
Sheki 1	58.3 fg	27.4 cde	64.2 ef	41.8 fg	49.7 ef	40.5 h	65.5 fg	62.5 def	51.2 G
Tale 38	70.2 c	26.3 de	76.8 bc	48.3 cd	54 cd	58.5 b	71.7 bc	73.5 b	59.9 CD
Zirve 85	61.9 ef	23.8 ef	68.9 d	44.4 defg	47.4 f	49.2 de	65 g	63.4 de	53 F
Average	61.5 C	27.2 G	69.8 A	47.3 F	52.7 D	49.0 E	66.9 B	66.6B	55.1
LSD (0.01) Variety	4.4	4	3.5	4	3.8	3	3	2.8	1.2
Coefficient of variation, %	4.2	8.7	3	5	4.3	3.7	2.7	2.5	3.9

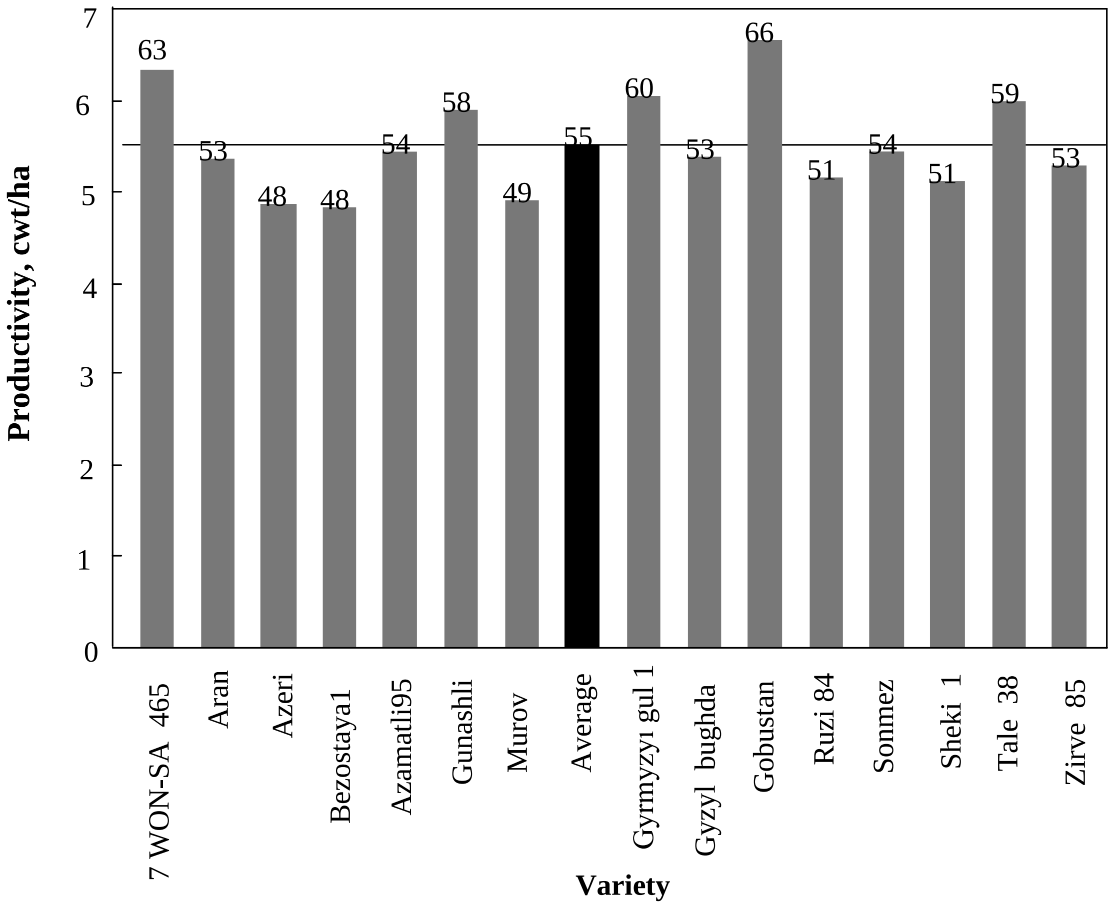


Fig. 1. The average productivity of the studied genotypes during all research years

Table 2. Stability parameters of the studied varieties related to productivity

Varieties	Productivity, cwt/ha	b		a	VC, %	R^2
		b	±Standard deviation			
7th WON-SA № 465	63.4	1.12	0.09	1.56	5.4	0.96
Aran	53.6	0.92	0.1	2.72	6.9	0.94
Azeri	48.7	0.9	0.13	-0.9	9.7	0.89
Bezostaya 1	48.3	0.84	0.16	1.72	12.7	0.82
Azamatli 95	54.4	1.13	0.2	-7.73	14.1	0.83
Guneshli	58.9	1	0.06	3.64	3.8	0.98
Murov 2	49.1	1.01	0.1	-6.44	7.9	0.94
Gyrmyzy gul 1	60.4	1.01	0.1	4.82	6.1	0.95
Gyzyl bughda	53.7	1	0.11	-1.22	7.6	0.93
Gobustan	66.4	1.19	0.12	0.64	6.8	0.94
Ruzi 84	51.6	0.87	0.12	3.75	8.5	0.9
Sonmez	54.2	0.84	0.08	7.8	5.4	0.95
Sheki 1	51.2	0.95	0.08	-1.04	5.5	0.96
Tale 38	59.9	1.18	0.08	-4.95	5.3	0.97
Zirve 85	53	1.04	0.05	-4.37	3.8	0.98
Average	55.1					

Bezostaya 1 productivity (cwt/ha)= 1.72+0.84*Environmental index
Azamatli 95 productivity (cwt/ha)= -7.73+1.13*Environmental index
Guneshli productivity (cwt/ha)=3.64+1.00* Environmental index
Murov 2 productivity (cwt/ha)=-6.44+1.01* Environmental index
Gyrmyzy gul 1 productivity (cwt/ha)=4.82+1.01* Environmental index
Gyzyl bughda productivity (cwt/ha)=-1.22+0.10* Environmental index
Gobustan productivity (cwt/ha)=0.64+1.19* Environmental index
Ruzi 84 productivity (cwt/ha)=3.75+0.87* Environmental index
Sonmez 01 productivity (cwt/ha)=7.80+0.84* Environmental index
Sheki 1 productivity (cwt/ha)=-1.04+0.95* Environmental index
Tale 38 productivity (cwt/ha) =-4.95+1.18* Environmental index
Zirve 85 productivity (cwt/ha)=-4.37+1.04* Environmental index

In these equations, the environmental index (EI) is the average productivity of all genotypes during a year. Based on the regression equations, stability parameters were determined by The Finlay-Wilkinson regression method (Finlay, Wilkonson, 1963) and the results are given in Table 2.

Parameters "a" and "b" (parameters required to assess the stability of the variety), the standard error of the "b" parameter, the coefficient of variation (VC, %), and the degree of significance - R^2 are given in the table. The parameters R^2 and VC are used to test the reliability of the regression equation without being directly related to the stability itself and have been proposed by Eberhart and Russell (Eberhart, Russell, 1966). R^2 and VC are a measure of the deviation between real values and values calculated by the regression equation. Low values of R^2, as well as high values of VC, indicate large deviations. Due to these deviations, the "a" and "b" values obtained for a variety as a result of regression analysis are considered to be unreliable. In our study, there was no problem with the reliability of the regression equations because all R^2 values were above acceptable limits (0.82-0.98).

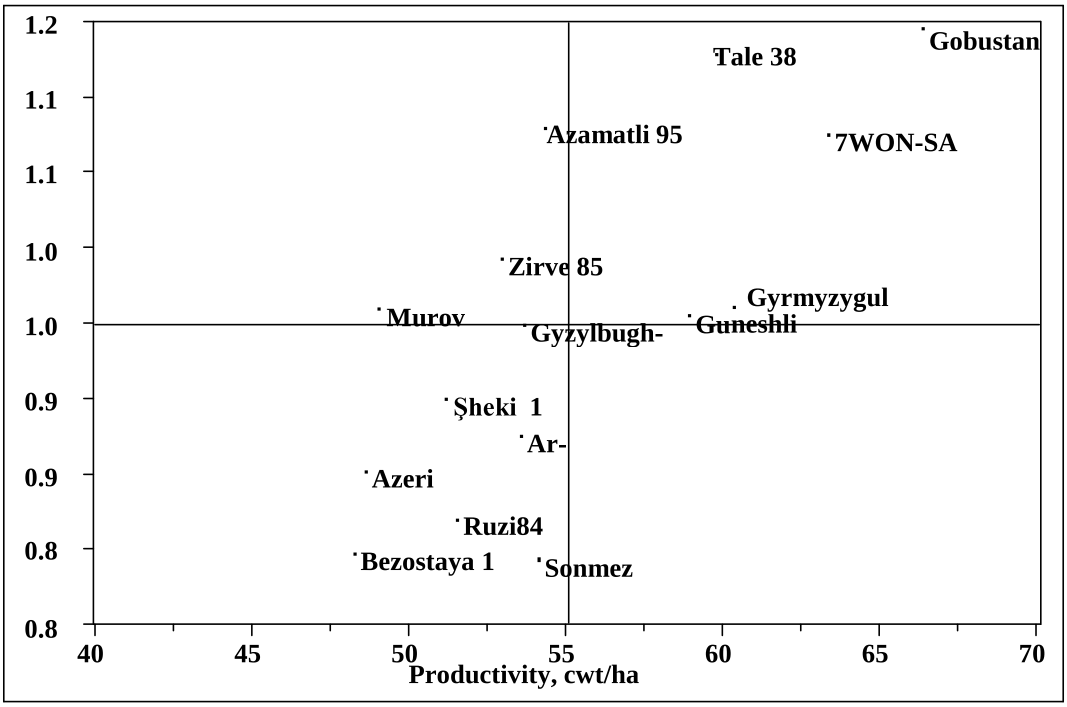


Fig. 2. Stability diagram constructed using productivity and "b" values.

The differences in the VC values were not considered significant because VC varied depending on the productivity levels and the values of R^2 were higher. The parameter "a" in the table is the expected productivity of the varieties at a point where the population average is theoretically zero. The parameter "b" indicates the increase in the productivity of varieties per unit of average productivity of the population in response to improved environmental conditions. High values of the "a" parameter indicate adaptability of the variety while the "b" values close to one indicate high stability of the variety. Therefore, values of "a" and "b" must be evaluated together when considering the yield stability of varieties. As seen in Table 2, 7thWON-SA №465, Aran, Bezostaya 1, Guneshli, Gyrmyzy gul 1, Gobustan, Ruzi 84, and Sonmez 01 varieties with positive values of the "a" parameter were considered as plants adapted to unfavorable conditions. Other studied varieties with negative values of "a" were unsuitable for unfavorable conditions. The varieties 7thWON-SA №465 and Gobustanın showing high values of the "b" parameter (1.12 and 1.19, respectively) were found to have wider adaptability. Azamatli 95 and Tale 38, which have negative values of "a", were considered to be highly adaptable to improved conditions, and as expected due to the high values of "b" (1.13 and 1.18), they were sensitive to adverse conditions. As mentioned above, the "b" values close to 1 indicate the high stability of the variety. The stability diagram constructed using the values of productivity and "b" is presented in Figure 2.

As seen in Fig. 2, Murov 2, Gyzyl bughda, Guneshli, and Gyrmyzy gul 1 varieties with "b" values close to one (1.01, 1.00, 1.00, and 1.01, respectively) were characterized as varieties with the highest yield stability. Figure 2 was divided into four sections to determine the adaptability of the varieties to different environmental conditions. The varieties with low adaptability under favorable conditions - Murov 2, Zirve 85, and Azamatli 95 were placed in the 1st section (upper left), Tale 38, Gobustan, 7thWON-SA №465, Gyrmyzy gul 1, and Guneshli showing high adaptability to favorable conditions - in the 2nd section (upper right). Gyzyl bughda, Sheki 1, Aran, Azeri, Ruzi 84, Bezostaya 1, and Sonmez 01 were placed in the 3rd section (lower left) as varieties badly adapted to unfavorable conditions. Section 4 (lower right) was to include varieties that were well adapted to unfavorable conditions, but none of the varieties studied matched this section. Taking into account the stability and plasticity of the studied varieties, it was concluded that Bezostaya 1, Ruzi 84, and Sonmez 01 having the "b" values much less than one (the main parameter determining stability and plasticity) were considered to be neutral varieties with the least plasticity (i.e. a type with wide adaptability). The productivity of these varieties was less reduced in unfavorable climatic conditions than in the case of ecologically plastic, i.e. intensive types. Since such varieties can be more cost-effective, it is advisable to cultivate them extensively. The varieties 7thWON-SA №465 and Gobustan having positive values of "a" and "b" showed adaptability to adverse conditions and high plasticity that determine their specific adaptation. These varieties not only increased their productivity by responding to high agrophone and favorable climatic conditions but also adapted to unfavorable conditions. Therefore, the cultivation of these varieties in areas with unstable moisture supply, such as Mountainous Shirvan, and in a high agrophone, creates conditions for good results. Tale 38 and Azamatli 95, which were not adaptable to unfavorable conditions, had negative values of "a", high values of "b", and were found to have high degrees of adaptability and high plasticity in response to improved conditions. It is expedient to cultivate these varieties in a high agrophone and well-watered conditions. The variety Gyrmyzy gul 1 manifested adaptability to unfavorable conditions (the "a" value was positive) and yield stability (the "b" value is close to 1), which indicated that it could be planted under unfavorable conditions. Taking into account other morphophysiological features and the high yield of this variety in favorable years, it is expedient to cultivate it in a high agrophone under irrigated conditions.

REFERENCES

Aliev J.A. (2000) Physiological bases of wheat breeding tolerant to water stress. *Proceedings of the 6th International Wheat Conference: Wheat in a global environment*. Hungary: Budapest, **9:** 693-698.

Aliev J.A. (2001) Diversity of photosynthetic activity of organs of wheat genotypes and breeding of high-yielding varieties tolerant to water stress. *Proceedings of the 12th International Congress on Photosynthesis*. Australia: Brisbane, p. 28-006.

Aliyev J. (2012) Photosynthesis, photorespiration and productivity of wheat and soybean genotypes. *Physiologia Plantarum*, **145:** 369-383.

Aliyev J.A., Huseynova I.M. (2014) Genotypic Variation for drought tolerance in wheat plants. P. Ahmad et al. (eds.). *Improvement of Crops in the Era of Climatic Changes*, Springer Science+Business Media, New York, **2:** 151-169; doi: 10.1007/978-1-4614-8824-8-6.

Eberhart S.A., Russell W.A (1966) Stability parameters for comparing varieties. *Crop Sci.*, **6:** 36-40.

Finlay K.W., Wilkonson G. N. (1963) The analysis of adaptation in a plant breeding programme. *J. Agric. Res*., **14:** 742-754

Mameev V.V., Nikiforov V.M. (2015) Evaluation of yield, adaptability, ecological stability and plasticity of winter wheat varieties in the Bryansk region conditions. *Bulletin of the Kursk State Agricultural Academy*, **No 7:** 125-129. (in Russ.). https://www.elibrary.ru/item.asp?id=28899333

Petrov L.K., Selekhov V.V. (2016) Results of the study of winter wheat varieties in the Nijegorod region conditions. *Agrarian Science of the Euro-North-East*, **2 (51):** 24-28. (in Russ.)

Samofalova N.E., Dubinina O.A., Samofalov A.P., Ilichkina N.P. (2019) The role of meteorological factors in formation of the productivity of winter durum wheat. Grain Economy of Russia, **5(65):** 18-23; https://doi.org/10.31367/2079-8725-2019-65-5-18-23) (in Russ.)

Sharma R.C., Morgounov A.I., Braun H.J., Akin B., Keser M., Kaya Y., Khalikulov Z., Ginkel M., Yahyaoui A., Rajaram S. (2012) Yield stability analysis winter wheat genotypes targeted to semi-arid environments in the International Winter Wheat Improvement Program. *International Journal of Plant Breeding* © Global Science Books, p. 7-13.

Sharma R.C., Rajaram S., Khalikulov S., Ziyaev Z., Hazratkulova S., Khodarahami M., Nazeri S.M., Belen S., Khalikulov Z., Mosaad M., Kaya Y., Keser M., Eshonova Z., Kokhmetova A., Ahmedov M.G., Jalal Kamali M.R., Morgounov A.I. (2013) Improved winter wheat phenotypes for Central and West Asia. *Euphytica*, **190:** 19-31; doi: 10.1007/s10681-012-0732-y.

Zhuchenko A.A. (2001) Adaptive system of plant breeding (ecological bases): monograph [in 2 volumes]. M.: Publishing house Rudn., **1:** 780 p. (in Russ.).

Nəmliklə stabil təmin olunmamış dəmyə şəraitində payızlıq yumşaq buğdanın məhsuldarlıq stabilliyi, plastikliyi və adaptivliyi

Cahangirov Atabəy Ağasoltan oğlu

AR KTN Əkinçilik Elmi-Tədqiqat İnstitutunun Qobustan Bölgə Təcrübə Stansiyası, Qobustan, Azərbaycan

2010/2011-2017/2018-ci vegetasiya illərində Dağlıq Şirvanın nəmliklə stabil təmin olunmamış dəmyə şəraitində payızıq yumşaq buğdanın 14 sortu və 1 xəttinin məhsuldarlığı, məhsuldarlıq stabilliyi, plastikliyi və sortun adaptivliyi öyrənilmişdir. Hidrometeoroloji göstəricilər əsasında 2010-2011, 2012-2013, 2016-2017 və 2017-2018-ci vegetasiya illərinin iqlim şəraitinin payılıq buğdanın inkişafı üçün əlverişli, 2014-2015 və 2015-2016-cı illərin iqlim şəraitinin orta, 2011-2012 və 2013-2014-cü illərin iqlim şəraitinin isə əverişsiz olduğu müəyyən edilmişdir. 2011, 2013, 2017 və 2018-ci illərdə bütün genotiplər üzrə orta məhsuldarlıq uyğun olaraq 61,3, 69,8, 66,9 və 66,6 sent/ha təşkil etməklə ən yuxarı səviyyədə olmuşdur. Məhsuldarlığa görə 2015 və 2016-cı illər orta, 2012 və 2014-cü illər isə aşağı səviyyədə olmuşdur. İllər üzrə orta məhsuldarlıq, vegetasiya ilində iqlim şəraitinin gedişi ilə uyğunluq təşkil etmişdir.

8 ilin ortalamasına görə Qobustan, 7thWON-SA №465 və Qırmızı gül-1 genotipləri uyğun olaraq 66,4, 63,4 və 60,4 sent/ha ilə məhsuldarlığın ən yuxarı, Azəri, Bezostaya-1, Murov-2, Ruzi-84 və Şəki-1 sortları isə ən aşağı nəticələrini göstərmişlər. Tədqiq edilən sortların stabillik və plastikliyini nəzərə alaraq belə bir nəticəyə gəlinmişdir ki, Bezostaya-1, Ruzi-84 və Sönməz-01 sortları plastikliyi ən az olan neytral tipə aid sortlar olaraq görülmüşlər (yəni geniş adaptivliyə malik olan tip). 7thWON-SA №465 və Qobustan sortları yüksək plastiklik göstərməklə spesifik adaptasiyaya malik olmuş, yüksək aprofona və əlverişli iqlim şəraitinə qarşılıq verərək məhsuldarlıqlarını artırmış, eyni zamanda əlverişsiz şəraitə də uyumluluq göstərmişlər. Ona görə də bu sortların Dağlıq Şirvan kimi nəmliklə stabil təmin olunmamış bölgələrdə və yüksək aqrofonda yetişdirilmələri yüksək nəticələrin alınmasına şərait yaradır. Tale-38 və Əzəmətli-95 sortlarının əlverişsiz şəraitə uyumlu olmamaları ilə yanaşı, yaxşılaşan şərtlərə yüksək səviyyədə qarşılıq verməklə dar adaptasiyaya və yüksək plastikliyə malik olmaları müəyyən edilmişdir.

Açar sözlər: *Payızlıq yumşaq buğda, dəmyə şərait, məhsuldarlıq, stabillik, plastiklik, adaptivlik*

Стабильность урожайности, пластичность и адаптивность озимой мягкой пшеницы в условиях богары с нестабильным влагообеспечением

Джахангиров Атабей Агасолтан оглу

Гобустанская зональная опытная станция НИИ земледелия МСХ АР, Гобустан, Азербайджан

Продуктивность, стабильность урожайности, пластичность и адаптивность 14 сортов и 1 линии озимой мягкой пшеницы исследовали в условиях богары Нагорного Ширваниа с нестабильным влагообеспечением в 2010/2011-2017/2018 вегетационных годах. По гидрометеорологическим показателям климатические условия 2010-2011, 2012-2013, 2016-2017 и 2017-2018 вегетационных годов признаны благоприятными для развития озимой пшеницы. Климатические условия 2014-2015 и 2015-2016 гг. – оценены как средние, 2011-2012 и 2013-2014 гг. - признаны неблагоприятными. В 2011, 2013, 2017 и 2018 годах средняя продуктивность по всем генотипам была самой высокой и составила 61,3, 69,8, 66,9 и 66,6 ц/га соответственно. В 2015 и 2016 годах продуктивность была средней, а в 2012 и 2014 годах – низкой. Средняя урожайность по годам соответствовала климатическим условиям вегетационногых годов. В среднем за 8 лет наибольшую продуктивность имели генотипы Гобустан, 7thWON-SA №465 и Гырмызы гюль-1, значения продуктивности которых составили 66,4, 63,4 и 60,4 ц/га соответственно. Самые низкие результаты показали сорта Азери, Безостая-1, Муров-2, Рузи- 84, и Шеки- 1. С учетом устойчивости и пластичности изучаемых сортов сделан вывод, что сорта Безостая- 1, Рузи- 84 и Сонмез- 01 относятся к нейтральному типу с наименьшей пластичностью (т.е. к типу с широкой адаптивностью). У генотипов 7thWON-SA №465 и Гобустан проявилась специфическая адаптация с высокой пластичностью и повышенной продуктивностью в ответ на высокий агрофон и благоприятные климатические условия, а также приспособленность к неблагоприятным условиям. Поэтому выращивание этих сортов в районах с неустойчивой влагообеспеченностью, таких как Нагорный Ширван, и в условиях высокого агрофона создает возможности для получения хороших результатов. Помимо неприспособленности к неблагоприятным факторам, сорта Тале-38 и Азаматли-95 обладают узкой адаптивностью и высокой пластичностью в ответ на улучшение условий.

Ключевые слова: *Озимая мягкая пшеница, богарные условия, урожайность, устойчивость, пластичность, адаптивность*

Journal of Life Sciences & Biomedicine, vol. 4(77), No 1, p. 13-21 (2022)

Factors providing adaptations of rose-ringed parakeet (*Psittacula krameri*) in the urban ecosystems of the city of Baku

***Karimov Tahir Arshad, Ahmadli Leyla Ilgar**

Institute of Zoology, Azerbaijan National Academy of Sciences, A.Abbaszade Str., Block 504, Passage 1128, Baku AZ1004, Azerbaijan
***For correspondence:** tahirornit@mail.ru, leyla.axmedli97@mail.ru*

Received: February 25, 2022; Received in revised form: March 03, 2022; Accepted: April 04, 2022

Rose-Ringed Parakeet is native to Southeast Asia and Africa. It is a new species in Azerbaijan's ornithofauna that are settled in the anthropogenic ecosystems of Baku. This article analyzes and interprets nesting, feeding behaviors and interspecific relationships that ensure the adaptation of this species to the urban ecosystems of Baku. Monitoring was conducted in the Baku Seaside National Park as a model area. Rose-Ringed Parakeet has been shown to exhibit high mobility and plasticity in both nesting and seasonal feeding strategies. That is, they select long-lived tall (20-40 m) trees with a trunk diameter of 0.6-1.3 m for successful nesting. They build their nests at a height of 4-6 m above the ground. The diameter of the entrance to the nests (8-10 cm) is suitable for the size of the bird's body. This protects the eggs and chicks from their natural enemies and increases the productivity of the nest (the number of chicks per pair). Another environmental factor that ensures the settlement of this species in Baku is the large number of feed facilities. Rose-Ringed Parakeet satisfies its need for food in all seasons by eating the fruits and seeds of 21 tree species. Both the above-mentioned and the lack of competition between species for nests, natural enemies, as well as human tolerance are the factors that created the conditions for the settlement of this species in the anthropogenic ecosystems of Baku.

***Keywords:** Rose-Ringed Parakeet (RRP) tree, nest, feed, adapt*

INTRODUCTION

Currently, the study of urban birds is one of the topical areas of ornithological research (Allen and Holling, 2010). This is due to the fact that a number of species are taken out of the natural habitat and span out in different ways (escapes from the cage and the zoo, the owner releases the bird from the cage, etc.) in other areas (mainly in urban parks, in gardens, etc.) as a result of human activities (introduction, commercial, smuggling) (Blackburn et al., 2015; Simberloff, 2009; Richardson, 2010; Parau et al., 2016). Of the 342 species of birds brought to Europe, 74 species have spread in the domestic environment through the above-mentioned ways and have formed numerous populations as a result of adaptation. Of these, 53 species are parrot species (Katsanevakis et al., 2012).

One of the species of parrots that are taken to regions outside its natural range and adapted to new natural conditions is the Rose-Ringed Parakeet. It is (ordo: *Psittaciformes* Wagler, 1830 - *Psittacula krameri* Scopoli, 1769) a native of Asia (Pakistan, India, etc.) and is located in Central Africa (Parau et al., 2016; Chalishkan, 2021). Rose-Ringed Parakeet is currently distributed in 71 countries around the world (Belausov, 2017).

It has been found that the number of Rose-Ringed Parakeetes (hereinafter referred to as RRPs) in Europe has increased rapidly since the 1960s at the expense of imported individuals (Strubbe and Matthysen, 2009). From 1981 to 2007, 231,048 RRPs were imported to 37 European countries (Jackson et al., 2015). In 2015, the number of RRPs in European countries was reported to be at least 85,120 individuals (Parau et al., 2016).

10.5281/zenodo.7239268

Available online 30 June 2022

RRP is a dendrophilic bird, nesting in trees and feeding on plant parts. Therefore, birds that escaped from cages and zoos and were released into the wild by their owners, settled in parks and gardens in cities with favorable climates and vegetation, forming large populations (> 500 individuals) (154 populations in 37 countries). (Parau et al., 2016). The role, number and adaptation features of RRP in urban ecosystems of Brussels (Strubbe et al., 2010: Weiserbs, 2010), London (Ballmer et al., 2013), Randstad (Claassen, 2014), Reyn-Heckar (Brown, 2009), Rome (Fraticelli, 2014), Istanbul, Ankara, Izmir (Parau et al., 2016; Caliskan, 2021), and in other cities have been studied to this day.

Baku is one of the cities inhabited by the RRP forming a natural population. In the years 2004 (Sultanov and Mammadova) and 2005 (Sultanov et al.), there were reports of registering this species in Baku. Another report states that RRP was illegally brought to Baku by a businessman in the 1990s (31 individuals) and released into the wild by the Customs (Belausov, 2017). It is noted that the number of RRPs in Baku increased from 21 to 32 individuals in 2003-2007 (Mammadova et al., 2008).

These authors provided only a small amount of information on the registration and number of RRPs. The role of environmental and anthropogenic factors, interspecific relationships, trophic ties, and limiting factors that ensure the adaptation of RRP to local urban ecosystems have not been analyzed or interpreted. Therefore, we aimed to study the role of floristic abandonment of phytocenoses, trophic conditions in the adaptation of RRP and the impact of biodiversity on the ecosystem.

MATERIALS AND METHODS

The study was carried out in the Seaside National Park in Baku (40°21′36″,49°50′13″). (Fig.1).



Fig. 1. General view and scheme of the Seaside National Park inhabited by RR.

Table 1. Number of trees and nests used by RRP for nesting

Type of tree	Height, m	Diameter, m	Tree lifespan, year	Number of slots	Height of the nest from the ground, m	Entrance to the nest, cm
Common ash, *F. excelsior*	30-40	0.7-0.9	200-250	5	4-6	8-10
Poplar pencil, *P.gracilis*	30-40	0.8-1	80-100	2	5-6	7-10
Black poplar, *P. nigra*	20-30	1-1.2	80-100	2	4-6	7-9
Oriental plane, *P.orientalis*	30-40	1-1.3	200-300	1	5	8
Horse-chestnut, *A.hippocastanum*	25-30	0.6-0.8	200-300	1	4	9

The research took place in the "Old Boulevard" area (3.5 km), which was established in 1909. The area of this park, selected as a model area, is larger than the area of the "Governor's Garden", "Philharmonic Garden", "Botanical Garden" and others, where the RRP was registered. In recent years, the territory of the park has been expanded and areas dubbed "New Boulevard", "White City", and "Flag Square" have been created. Over 100 species of long-lived trees and shrubs imported from Italy, Turkey, Mexico, Spain, France, Germany, the United States, Australia, Africa, Argentina, and other countries were planted and adapted to the local climate in both these areas and in the "Old Boulevard". Seaside National Park is one of the places of recreation and tourism. There are also many commercial, entertainment, recreation and sports facilities, as well as highways and tall residential buildings nearby.

Objects of research - birds, trees, shrubs, nests, and food eaten by birds. The type, height (roughly), diameter (with a measuring tape), and the number of trees nested by the RRP (Mammadova, 2010) in the park were defined. The number of nests in the trees, the height from the ground, the diameter and depth of the entrance (with a tape measure) were studied. In addition, the type of trees and shrubs they eat in all seasons (fruits, seeds, shoots, leaves, etc.), the shape of the fruit, the ripening period (Mammadova, 2010), the daily and seasonal feeding behavior of birds were studied as well. Observations and records were made in April, September, December of 2021 and January 2022. Route and stationary observation methods were utilized.

Intergenerational ties also influence the adaptation of RRP to phytocenoses. Therefore, nesting and diet competition behaviors between birds and animals living in the park and the RRP were studied. RRP's self-defense and aggressive behaviors were studied as well.

RESULTS AND DISCUSSION

Note that, only the registration and number of RRPs have been indicated in both the Seaside National Park and other gardens, while the characteristics of phytocenosis adaptation have not been studied (Sultanov and Mammadova, 2004; Sultanov et al., 2005; Mammadova et al., 2008). Eleven pairs (22 individuals) were found nesting in the park. 50-60 RRPs are recorded in the park area, along with young individuals and individuals coming from other areas to feed. Considering this, we have studied the role of factors providing the adaptation of RRP to the phytocenoses of the Seaside National Park. It was identified that the species composition of trees and shrubs has a significant impact on the settlement and number of dendrophilic RRP in phytocenosis. This is because RRP uses different types of trees and shrubs for nesting, feeding, spending the night, and relaxing. For these purposes, RRPs prefer different trees and shrubs belonging to domestic phytocenoses in different geographical areas (Mohammad et al., 2015; Chalishkan, 2021).

It was found that RRPs choose nesting sites by relying on the characteristics of the trees such as altitude, the diameter of the trees, age, size, and depth of the nest cavity. In the Seaside National Park, RRPs use the trees listed in the table for nesting (Table 1). All 11 nests we recorded are located in 5 species of trees. 45.45% of the 11 nests are located in the *Common Ash* tree. *Poplar*

pencil (18.18%), *Black poplar* (18.18%), *Oriental plane* (9.90%), and *Horse-chestnut* (9.90) trees are taken the next places on the list. Other parameters of trees and nests in the park are given in Table 1.

If we compare the results of our and other authors, we can see differences and similarities. For comparison, in Germany, the majority (57.0%) of RRP nests are located in the Plantain leaf maple (*Acer pseudoplatanus*) tree with a trunk diameter of 0.8-1.50 m (Chaika et al., 2011). In India and Africa, the homeland of the RRPs, nests are located at 9-27 and 5-14 m above the ground. In Pakistan, the RRP trunks nests in 15 different species of trees with a diameter of 0.6-2.15 meters. The entrance to the nest is 5.5-20.3 cm and is located 4-6 m above the ground (Mohammad, 2015). Differences in the height of the nests from the ground, the density of tree trunk, and the diameter of the nest entrance are due to the fact that different species of trees in different areas do not grow at the same rate and they gradually age. This process is significantly influenced by the soil surface, climate, diseases, and parasites of the area where the trees are located.

It has been identified that RRPs prefer long-lived, old, and tall trees for nesting. Other studies also confirm it (Prajapati and Prajapati, 2012; Renton et al., 2015). Considering that the "Old Boulevard" area of the park was created in 1909, we can say that the trees, where the nests are located (they are trees of local nature) are over 100 years old. The location of the nest in a long-lived tree allows them to use it for a long time. As the tree ages, cracks and fissures form in its trunk. It is easier to build a nest there. Such cracks allow for 2-3 nests in a tree. There are 2-3 nests per tree in Pakistan (Mohammad, 2015) and 9 nests in Germany (Chaika et al., 2011).

We recorded only one nest in one tree in the Seaside National Park. The holes in the wood are made by the RRP itself. The diameter of the entrance is according to the body size. Stray cats in the park enter the nest and try to eat the egg and nestlings. However, the small entrance to the slot does not allow it. The absence of snakes in the park also contributes to safe nesting. The depth of the nests is 27-32 cm. At the bottom of the nest, the birds lay leaves and feathers. We recorded 1 hole in each slot. The hole is used for entry and exit (Fig. 2).

The scientific literature indicates that older and more experienced couples prefer trees with a diameter of 2 m for nesting (Mohammad, 2015). If we consider that RRP nested in the trees with a diameter of 0.6-1.3 m (Table 1) and 19 individuals were registered for the first time in the Seaside National Park in 2004 and 2 individuals in 2006 (Mamadova et al., 2008) we can say that the population is younger.

It was found that the adaptation of RRPs to the Seaside Park had a significant impact on the diet stocks of the phytocenosis, in addition to the factors mentioned above. This is because stocks in the phytocenosis affect the time of RRP nesting and nest productivity (number of nestlings per pair). Therefore, the presence of feed objects is important in the RRP's strategy of nesting site selection. According to the literature, parrots also follow the ripening stage (phenology) of the fruits of individual trees and use them in feeding strategies. Such a selection strategy increases the probability of success in reproduction (Renton et al., 2015).

More than 3,000 trees, shrubs, and grasses have been planted in Seaside Park. Here, we recorded that RRPs fed with soft parts of 22 species of trees and shrubs (i.e., shoots, flowers, fruit stems) and hard seeds (Table 2).

It is related to the blossoming of trees and shrubs in various months, ripening of their seeds and fruits, and remaining duration on the tree. In spring and summer, RRP feeds on vegetative (leaf) and generative (flower) shoots of 12 species of trees and shrubs (Table 2). RRP feeds on leaves and flower shoots both simultaneously (i.e. when germinating at the same time) and separately (for example, *Cotoneaster acuminatus; Aésculus hippocástanum; Quercus ilex*) (Table 2). The trees and shrubs in the park bloom in different months. Commencing from March (Laurus nobilis) until October (*Vítex ágnuscástus) Vítex ágnuscástus*) the development of vegetative and generative organs allows the RRP to feed on the soft parts of plants in the spring and summer. Birds eat different parts of the flower (pistil, perianth, stamen, petal). Starting from February (*Cupressus arisonica*) until January (*Pinus pinea)* the seeds and fruits ripen. As a result, the feed ration of the RRP becomes richer in the fall months. During this period, RRP feeds on 20 species of trees and shrubs and seeds (Table 2).

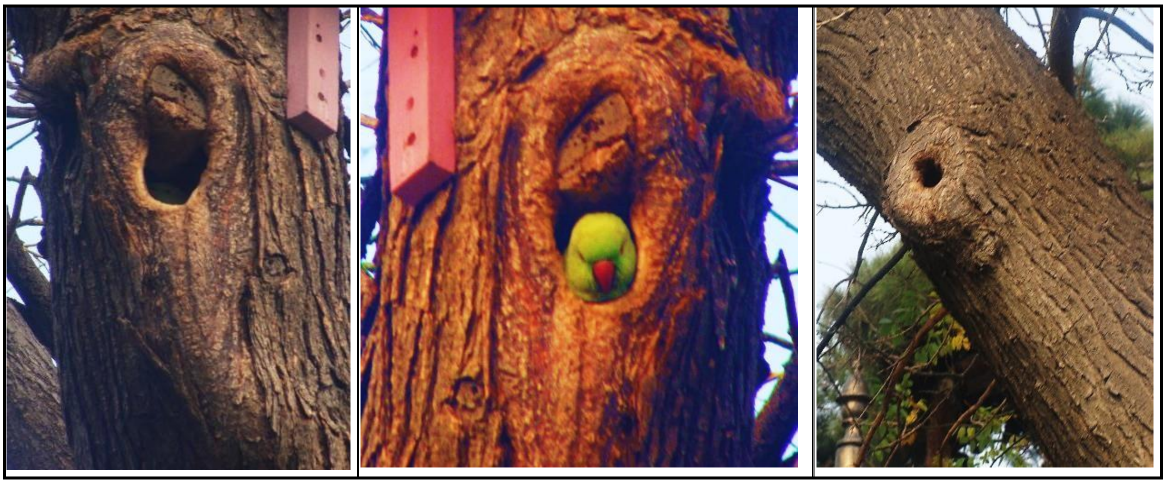


Fig. 2. Nests built and used by RRP on various trees.

Table 2. Trees and shrubs, containing diet sources of the RRPs in the Seaside National Park

Trees and shrubs	Number, pieces	Diet spectrum		
		flower and leaf shoots	seeds	fruit pulp
Styphnolobium japonicum, *Sofora Yaponica*	12	+ VII-VIII	+ IX-X*	
Laurel, *Laurus nobilis*	6	++ III-V		+ IX
Olive, *Olea europaea*	102	++ V-VI		+ IX-X*
Eastern thuja, *Biota orientalis*	46		+IX-XI	
Cilver crest, *Pinus pinea*	21		+ X-XII	
Chaste tree, *Vítex ágnuscástus*	30	++ VI-X	+X-XI	
Arizona cypress, *Cupressus arisonica*	22		+II-III	
European dwarf,*Cercis siliquastrum*	19	++ IV-V	+VIII-IX	
Finik, *Phoenix dactylifera*	20			+IX-XI
Yukon Belle, Pyracantha angustifolia	75		+ VIII	+ VIII
Cotoneaster, *Cotoneaster acuminatus*	10	+ IV-V	+ VIII	
Chestnut, *Aésculus hippocástanum*	31	+ V		+ VIII-IX
Holm qak, *Quercus ilex*	4	+ IV	+ X*	
Oriental plane, *Platanus orientalis*	7		+IV-VI	
Black poplar, *Pópulus nígra*	43	++ IV-V		
Poplar pencil, *Populus gracilis*	30	++ IV-V		
Common barberry, *Berberis vulgaris*	17	-		+IX-X
Common ash, *Fraxinus excelsior*	67	++ IV-V	+ VIII-IX*	
Pino, *Pinus eldarica*	80		+ X*	
Hornbeam, *Carpinus betulus*	32	++ IV-V		+ X
Notkins cypress, *Cupressus nootkatensis*	11		+ IX-X	

Fruits and seeds remain in the park for a long time on 163 trees and shrubs *(Sofora Yaponica; Quercus ilex; Fraxinus excelsior; Pinus eldarica; Olea europaea)*. These fruits and seeds form fodder facilities from October to March. In general, the share of seeds in the feed rations of RRP is higher. We identified that the RRP ate more of the Hornbeam (*Carpinus betulus)* fruit (nut) more and enthusiastically. The shells of the walnuts they eat are poured under the tree. Relying on these shells, it is possible to define the number of nuts they eat. They also fly to a nearby "botanical garden" to eat the fruit of this tree. Starting from February (*Cupressus arisonica*) until January (*Pinus pinea)* the seeds and fruits ripen. As a result, the feed ration of the RRP becomes richer in the fall months. During this period, RRP feeds on 20 species of trees and shrubs and seeds (Table 2). Fruits and seeds remain in the park for a long time on 163 trees and shrubs *(Sofora Yaponica; Quercus ilex;*

Fraxinus excelsior; Pinus eldarica; Olea europaea). These fruits and seeds form fodder facilities from October to March. In general, the share of seeds in the feed rations of RRP is higher. We identified that the RRP ate the Hornbeam (*Carpinus betulus)* fruit (nut) more and enthusiastically. The shells of the walnuts they eat are poured under the tree. Relying on these shells, it is possible to define the number of nuts they eat. They also fly to a nearby "botanical garden" to eat the fruit of this tree.

We did not observe RRPs eating grass on the ground. The variety of diet objects (seeds, pine and oak cones, nuts, acacia beans, walnut, berries, and others) ripening at different times and remaining in trees and bushes for a long time (fruits are not harvested in the park) allows an RRP to find diet in all seasons. In the autumn and winter months, RRP's diet rations are richer (Table 2). In spring and summer, shoots, flowers, leaves, fruits, and seeds that remain on trees and shrubs for a long time form the diet of RRP.

Other ornithologists also report that RRPs change their feeding patterns from fruit or seeds to flowers at different times of the year. It is noted that the ration of the RRPs is formed from 7-26 plant species depending on the seasons. Ornithologists state that RRP demonstrates high mobility and flexible behavior in diet search and seasonal feeding strategies (Blanco et al., 2015). This is a universality that allows the RRPs to span out to the most diverse geographical areas.

It should also be noted that the presence of trees and shrubs of African and Asian origin (i.e., belonging to their historical homeland) in the Seaside Park (*Phoenix dactylifera*; *Olea europaea*, *Laurus nobilis*, *Vítex ágnus cástus*, *Pyracantha angustifoli*, *Quercus ilex*, *Taxus baccata* other) allows them to feed on traditional diet objects. One of the factors ensuring the settlement of RRPs in the park is the lack of competition between species and nests. One reason for this is the large number of tree species suitable for nesting. Another reason is that other species (Picidae, Sitidae, and others) that nest in tree crevices and holes do not breed in the park site. RRPs, which are better adapted to anthropogenic ecosystems than other bird species, are able to use nesting sites. In addition to RRPs, Rock dove (*Columba livia*) and Collared dove (*Streptopelia decaocto*) nest and feed in the park. These species are not considered RRP's diet competitors. Although the main feeding competitor was Hooded crow (*Corvus cornix*) we did not see any conflict over diet. Instead, we recorded that they feed on the same tree (Fig. 3).

Currently, the domestic population of this species has been formed in Baku. RRP is a new species in the Azerbaijani ornithofauna. Therefore, it is of great importance to predict the impact and role of RRP as an invasive species in the biodiversity of Azerbaijan. In order to clarify this issue, let's pay attention to the numerical dynamics and dispersion of the RRP population.



Fig. 3. There was no feed competition between RRPs and Hooded crow (*Corvus cornix*) on the same tree.

The number of this species in Baku ecosystems was 21 in 2003, 36 in 2004, 28 in 2005, 27 in 2006, and 32 in 2007 (144 individuals in total) (Mammadova et al., 2008). As can be seen, the number has increased in fluctuation type over 5 years. It is known that abiotic (weather conditions) and anthropogenic factors influence the processes in the population that regulate the dynamics of numbers (Neverova and Frisman, 2020). The following factors influenced the change in the number of RRPs in Baku under fluctuations. In some years, strong and cold winds, rainy, and frosty weather conditions have a negative impact on the number of pairs involved in reproduction and the beginning of reproducetion. The number of eggs laid in the nest is also decreasing. Currently, a maximum of a1 or 2 eggs is recorded in each nest. That is, nest productivity (the number of chicks given by a pair) is not higher. The anthropogenic factor is the illegal detention and sale of baby birds. This factor leads to a change in the sex ratio in the population and a decrease in the number of breeding pairs. At present, 200-250 individuals are registered in Baku. Due to the lower reproductive capacity of the population, the dispersion of RRP to other parts of the country is not observed. However, as an invasive species, RRP needs to be studied regularly and appropriate predictions made.

CONCLUSION

The RRP population in the Seaside National Park (over 100 years old) is young in terms of the time period. The optimal ecological conditions of the ecosystem and the ethological characteristics of these birds had a significant impact on the settlement of the species that are native to Southeast Asia and Africa in the park. Environmental factors include the richness of the floristic composition of the ecosystem, the abundance of tree species for nesting, overnight protection, nesting, lack of enemies in diet grounds and interspecific competition, favorable trophic conditions, and wild climate. Ecological flexibility of RRP (i.e., acceptance to changes in environmental conditions), tolerance, fearless closeness with humans, colonial nesting, feeding, and protection are also ethological factors that ensure the adaptation of this species to urban ecosystems.

ACKNOWLEDGMENTS

We express our gratitude to the staff of the Baku Seaside National Park for their assistance in collecting materials.

REFERENCES

Məmmədov T. (2010) Abşeron ağacları və kolları. Bakı: Elm və təhsil, 468 s.

Sultanov E.Q., Məmmədova S.İ. (2004) Quşlar uçur ... Bakı: *Xəbər qəzeti*, **№2:** 14.

Sultanov E.Q., Məmmədova S.İ., Kəmərli R.A., İsmayılova S.M. (2005) Nəbatat Bağının Avifaunası. Bakı: *Ekologiya, fəlsəfə, mədəniyyət*, s. 111-113.

Белаусов Е.М. (2017) О встречах попугая Крамера (Psittacula krameri) в предгорьях Таласского Алатау. *Российский орнитологический журнал*, **26(экспресс-выпуск 1463):** 2627-2628.

Мамедова С.И., Исмаилова С.М., Камарли Р.А. (2008) К экологии попугая Крамери в г. Баку. *Труды Азербайджанского общества зоологов*, **1:** 700-703.

Allen C.R., Holling C. (2010) Novelty, adaptive capacity, and resilience. *Ecol. Soc.*, **15:** 24.

Ballmer D.E., Gillings S., Caffrey B.J., Swann R.L., Downey I.S., Fuller R.J. (2013) Bird Atlas 2007-2011: Breeding and Wintering Birds of Britain and Ireland. Thetford: BTO Books.

Blackburn T.M., Lockwood J.L., Cassey P. (2015) Influence of numbers on invasion success. *Mol. Ecol.*, **24:** 1942-1953; doi: http://dx.doi.org/10.1111/mec.13075.

Blanco G., Giraldo, F., Jimenez Rojas A., Denes F. (2015) Parrots as key links in the structure and functioning of the ecosystem. *Ecology and evolution*, **5 (18):** 4141–4160. doi: 10.1002/ece3.1663.

Brown M. (2009) Die Bestandssituation des Halsbandsittichs Psittacula Krameri in the der Rhein-Neckar-Region (Baden-Württemberg, Rhineland-Pfalz, Hessen), 1962-2008, im Kontext der gesamteuropäischen Verbreitung. *Vogelwelt*, **130:** 77-89.

Chaika C., Michael P., Brown P. (2011) Resource utilization by non-native ringed parrots (*Psittacula krameri*) and indigenous starlings

(*Sturnus vulgaris*) in Central Europe. *Open Ornithological Journal,* **5:** 17-22; doi: 10.2174/1874453201104010017.

Chalishkan O. (2021) Parakeets of Ankara: rose-ringed parakeets (*Psittacula krameri*) as exotic species and anthropogenic habitats in Ankara. *International Journal of Geography and Geographical Education,* **44 (1):** 445-460. https://doi.org/10.32003/igge.854992.

Fraticelli F. (2014) In pink-ringed parrot *Psittacula krameri* in a city park: demographic trend of interspecies relationship and feeding preferences (Rome, Central Italy). Avocetta 38: 23-28.

Jackson H., Strabbe D., Tollington S., Pris-Jones R., Matthisen E., Grumbridge J.J. (2015) Ancestor Origins and Global Invasion. invasive birds are correlated with climate and the influence of the bird trade. *Mol. Ecol.,* **24:** 4269-4285; http://dx.doi.org/10.1111/mec.13307).

Katsanevakis S., Bogukarskis K., Gatto F., Vandekerkhove J., Deriu I., Cardosu A.S. (2012) Establishment of the European Alien Species Information Network (EASIN): A new approach to exploring data on distributed alien species. *Bio. Invasions Rec.,* **1:** 235-245; http://dx.doi.org/10.3391/bir.2012.1.4.01.

Mohammad W., Irfan A., Tanvir H. (2015) Nesting behavior of rose-ringed parakeet in southern Panjab in Pakistan. *Sci. Int. (eLahor),* **27 (5):** 4255-4260.

Neverova Q.P., Frisman E.Y. (2020) Population dynamics regimes with non-overlapping generations considering genetic and stage structures. *Computer research and modeling,* **12(5):** 1165–1190; doi: 10.20537/2076-7633-2020-12-5-1165-1190

Pârâu D., Strubbe E., Morfi M. et al. (2015) Rose-ringed parakeet populations and numbers in Europe: A complete overview. T*he Open Ornithology Journal,* **9 (1):** 1-13; doi: 10.2174/1874453201609010001.

Prajapati S., Prajapati R. (2012) Observations on Hovering. Rose-ringed parrot (*Psittacula krameri*) habitat. *Life Sciences Pamphlet*s, **7:** 56-62.

Simberloff D. (2009) The role of propagule pressure in biological invasions. *Annu. Rev. Ecol. Evol. Syst.*, **204:** 81-102.

Strubbe D., Matthissen E., Graham C.H. (2010) Assessing the potential impact of the invasive round-necked parrots *Psittacula krameri* on the native nuthatch *Sitta europeae* in Belgium. *J. Appl. Ecol.,* **47:** 549-57. doi: 10.1111/j.1365-2664.2010.01808.x.

Weiserbs A. (2010) Especes invasives: Le cas des Psittacides en Belgique. Morbidity, risk and event assessment. *Aves*, **47:** 21-35.

Bakı şəhərində məskunlaşan yaxalıqlı tutuquşunun *(Psıttacula kramerı)* eko-etoloji xüsusiyyətlərinin öyrənilməsi

Kərimov Tahir Ərşad oğlu, Əhmədli Leyla İlqar qızı

AMEA-nın Zoologiya İnstitutu, Bakı, Azərbaycan

Yaxalıqlı tutuquşunun vətəni Cənubi-Şərqi Asiya və Afrikadır. Azərbaycan ornitofaunasında yeni növdür. Bakı şəhərinin antropogen ekosistemlərində məskunlaşmışdır. Məqalədə bu növün Bakı şəhərinin urboekosistemlərinə adaptasiyasını təmin edən yuvalama, yemlənmə davranışları və növlərarası münasibətləri analiz və şərh edilmişdir. Monitorinq model ərazi kimi Bakı Dənizkənarı Milli Parkda aparılmışdır. Müəyyən edildiki, Yaxalıqlı tutuquşu həm yuvalama yeri seçmə, həmdə mövsümi yemlənmə strategiyalarında yüksək mobillik və plastik davranış nümayiş etdirirlər. Yəni yuvaqurmanın uğurlu olması üçün uzunömürlü hündür (20-40 m), gövdəsinin diametri 0.6-1.3 m olan ağacları seçirlər. Yuvalarını yerdən 4-6 m hündürlükdə qururlar. Yuvaların girişinin diametri (8-10 sm) quşun bədən ölçüsünə uyğun olur. Bunlar yumurtanı, balanı təbii düşmənlərindən qoruyur yuva məhsuldarlığını (1 cütün

verdiyi bala sayı) artırır. Bu növün Bakıda məskunlaşmasını təmin edən digər bir ekoloji amil yem obyektlərinin çox olmasıdır. Yaxalıqlı tutuquşu bütün fəsillərdə yemə olan tələbatını 21 növ ağacın meyvısini, toxumunu yeməklə ödəyir. Həm qeyd etdiklərimiz, həmdə növlərarası yuva, və yem rəqabətinin, təbii düşmənlərinin olmaması, həmçinin insana tolerantlığı, bu növün Bakının antropogen ekosistemlərində məskunlaşmasına şərait yaratmış amillərdir.

Açar sözlər: *Yaxalıqlı tutuquşu, ağac, yuva, yem, adaptasiya*

Факторы, обеспечивающие адаптацию ожерелового попугая (*Psittacula krameri*) к урбоэкосистеме на примере города Баку

Керимов Тахир Аршад оглу, Ахмедли Лейла Илгар кызы

Институт зоологии НАН Азербайджана, Баку, Азербайджан.

В статье анализируются и интерпретируются гнездовое и пищевое поведение, ~~и~~ также межвидовые отношения, обеспечивающие адаптацию ожерелового попугая (*Psittacula krameri*) к Баку, как к самой крупной урбоэкосистеме Азербайджана. Мониторинг проводился в Бакинском приморском национальном парке, использукмом в качестве модельной территории. Было обнаружено, что попугай проявляет высокую подвижность и пластичность как в стратегиях гнездования, так и в стратегиях сезонного кормления. Показано, что для успешного гнездования ожереловые попугаи выбирают долгоживущие высокие (20-40 м) деревья с диаметром ствола 0,6-1,3 м. Свои гнезда строят на высоте 4-6 м над землей. Диаметр входа в гнезда (8-10 см) соответствует размеру тела птицы. Гнезда защищают яйца и птенцов от их естественных врагов и повышают продуктивность гнезда (количество птенцов, приходящееся на 1 пару). Еще одним экологическим фактором, обеспечивающим расселение этого вида в Баку, является большое количество кормовых объектов. Попугай удовлетворяет свои потребности в пище в любое время года, поедая плоды и семена 21 видов деревьев. Все вышеперечисленное, а также отсутствие межвидовой, гнездовой и кормовой конкуренции, естественных врагов и толерантность человека являются факторами, создавшими условия для расселения этого вида в антропогенных экосистемах города Баку.

Ключевые слова: *Ожереловый попугай, дерево, гнездо, корма, адаптация*

Journal of Life Sciences & Biomedicine, vol. 4(77), No 1, p. 22-27 (2022)

New distribution areas of some species (*O. altissima, O. michauxii* and *O. buhseana*) of sainfoin (*Onobrychis* Mill.) in the flora of Azerbaijan and their bioecological characteristics

Azizkhanli Kharibul Mammadkhan, *Asgarov Aydin Musa

Genetic Resources Institute, Azerbaijan National Academy of Sciences, 155 Azadlig Ave., Baku AZ1106, Azerbaijan
***For correspondence:** askerov1@mail.ru*

Received: March 31, 2022; Received in revised form: April 01, 2022; Accepted: April 08, 2022

The paper describes 8 new distribution areas of 3 species (*O. altissima, O. michauxii,* and *O. buhseana*) of sainfoin, their bioecological and phytocenological characteristics. The new distribution areas cover the northern part of the Lesser Caucasus, Talysh, and Nakhchivan regions. The goal is to clarify the distribution areas of the sainfoin species in the flora of Azerbaijan and to distinguish for selection species more resistant to drought and frost. The article provides a new distribution map of the collected species of sainfoin, a table with environmental information and photos.

Keywords: *Sainfoin, population, bioecology, family, genus, species, areal*

INTRODUCTION

The study of the forage reserves in the hayfields and pastures of our republic, identification of their species composition, and the natural populations in terms of conservation of the ancestor crops is very important.

The species belonging to the leguminous (*Fabaceae*) family are of high significance. One of the most important forage genera mentioned in the family is sainfoin (*Onobrychis* Mill.). *Onobrychis* Mill. (Miller, 1754), tribe *Hedysareae* (Lock, 2005, Polhil, 1981) has 173 species in the world and 25 species (Asgarov, 2016) of them are widespread in Azerbaijan. Some of them are endemic to the Caucasus. A number of investigations of this genus were carried out (Sirjaev, 1925, Yildiz, 1999).

The species of *Onobrychis* Mill. can be used both forage crops and initial material for drought and cold-resistant, productive cultivated varieties growing (Khinthibidze, 1960). In Azerbaijan 3 species (*O. viciifolia, O.arenaria, O. transcausasica*) of *Onobrychis* are cultivated.

MATERIALS AND METHODS

During the expeditions, descriptor data, herbarium specimens and seed samples of plants were collected and a GPS device was used to record the coordinates. "Flora of Caucasus" (Grossheim, 1952), "Flora of Azerbaijan" (Tamamshyan, 1954), "The plant world of Azerbaijan" (Asgarov, 2016) were used as literary sources. The collected herbarium specimens were sent to the Herbarium Funds of the Genetic Resources Institute and the Institute of Botany of ANAS and seed samples were sent to the Genebank.

RESULTS AND DISCUSSION

During the expeditions conducted in 2013, 2018, 2019, and 2021, the employees of the Department of Ecobotany and Taxonomy of the Genetic Resources Institute of ANAS revealed the new distribution range of three species of sainfoin (*O.altissima*, *O.michauxii*, and *O.buhseana*) in Lerik, north part of Lesser Caucasus and Nakhchivan regions (Table 1, Fig.1).

***O. altissima* Grossh.** - High sainfoin. It belongs to the *Eubrychis* section. Typus: Prov. Tiflis, distr. Gori. Окр. Баржоми спуск к р. Кура et с. Гамма. Leg: W. Kozlowsky, 1923, (TBI).

O. altissima is very close to the cultivated *O. viciifolia* Scop. species and may have its wild ancestor. In 1954, Tamamshyan studied the genus *Onobrychis* Mill. of the Flora of Azerbaijan and

10.5281/zenodo.7239419
Available online 30 June 2022

recorded that the species of *O. altissima* is close to *O. inermis* and distinguished only by the distribution area (Tamamshyan, 1954). Also in our opinion, *O. altissima* is very close to *O. inermis*. But they differ from each other in the length of the standard (in *O.inermis*standard shorter than keel).

O. altissima reaches the height of 90 cm, the stems are straight, almost rounded at apex, lower leaves long-petioled, with 6-8 pairs of oblong-oval or elliptic leaflets 12-30 mm long and 5-7 mm wide, the corolla is 10-11 mm long, the size of standard equals the keel. Pods are 5-7 mm long, 1.5-2 mm wide, crest, no prickles. Inflorescence occurs in June-July and fruit ripening in July-August. It is found in meadows, in shrubs, on rocky and sloping areas of the middle and subalpine zone.

According to literature data, *O. altissima* was found in Armenia (Tamamshyan, 1954), Turkey (Hedge, 1970), Iran (Ranjbar, 2010), and the Diabars (Talysh) region of Azerbaijan. Herbarium (BAK) material was collected in 2015 by Gasymova Sh. from the village of Avakhil in the Shamakhi district. As a result of our research, this species was collected in 2013 from 3 biologically rich regions of the Lesser Caucasus - the village of Yeni Zod of the Goy-Gol district, the village of Zakhmatkand of the Gadabay district, and Yasamal pass of the Shamkir district.

Table 1. New distribution areas of *O. altissima, O. Michauxii,* and *O. buhseana*

Pop-on №	Collection area	Coordinate	Collection date and route
***O. altissima* Grossh.**			
1	Goy-Gol district, Yeni Zod village	N40°28'898" E046°20'808"	V_{1-1} 29.06.2013
2	Nakhchivan MR, Shahbuz district, Bichanak village, Batabat pasture, Goshagoz Bridge	N39°53'524" E45°79'866"	NVSh-4 01.07.2021
3	Shamkir district, on the side of the Yasamal road	N40°48'225" E045°57'760"	Y_{1-3} 27.06.2013
4	Gadabay district, Zakhmatkand village	N40°41'821" E045°49'940"	G_{1-2} 27.06.2013
***O. michauxii* DC.**			
5	Lerik district, Hoveri village	N38°41'334" E48°23'588"	ST_1 18.06.2019
6	Lerik district, Galasar village, Syxabin sanctuary	N38°41'485" E48°23'905"	ST_4 19.06. 2019
7	Lerik district, Galasar village, Syxabin sanctuary	N38°41'485" E48°23'905"	Z_6 05.07.2018
***O. buhseana* Bunge ex Boiss.**			
8	Lerik district, Nimakesh village	N38°39'549" E48°22'095"	ST2 18.06.2019

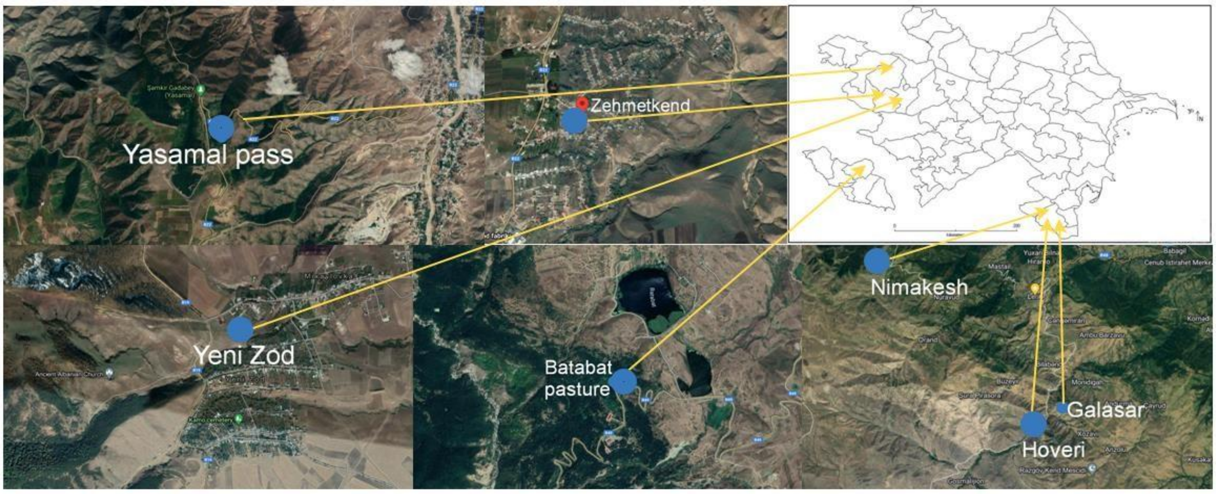


Fig. 1. Distribution map of *O. altissima*, *O. michauxii* and *O.buhseana.*



a **b**

Fig. 2. General view (a) and populationview (b) of *O. altissima.*

Although, 16 of 25 species of the genus *Onobrichis* Mill. are spread in the territory of Nakhchivan, which differs from other physical and geographical regions of Azerbaijan in its heavy continental climate, species *O. altissima* was not found in these territories till now. It was revealed around the Batabat pasture of the village of Bichanakin Nakhchivan in 2021 (Fig.2a, 2b).

***O. michauxii* DC.** – Misho sainfoin. Belongs to the *Hymenobrychis* section, it was described from Iran. Its type is located in Paris.

The roots of *O. michauxii* are polysepalous and quite thick. The long stem consists of 7-10 pairs of leaflets. Pods are small, 10-12 mm wide, densely long-villous-tomentose with short prickles concealed by pubescence. Inflorescence occurs in May-June and fruit ripens in June-July. It is found in dry slopes, rocks, rocky, sloping areas, and shrubs in the low and moderate-high belts.

According to the literary data, the species of *O. michauxii*are found in Iran (Ranjbar, 2012), Armenia (Tamamshyan, 1962), the Lesser Caucasus and the Nakhchivan (Tamamshyan, 1954) region of Azerbaijan. Herbarium data (BAK) was collected in 1936 in the Goychay district, in 1937 in the village of Maraza of the Shamakhi district and in 2018 and 2019 in the territory of the village of Hoveri and Galasar of Lerik district as well (Fig.3).

***O. buhseana*Bunge ex Boiss.** – Buze sainfoin. Refers to the section *Heliobrychis*, described from Tabriz. Type is in Paris.

Leaves (2) have 3-4 pairs of leaflets. The standard is rounded at the apex, keel, and wings are glabrous. Pods 6-8 mm wide, semiglobular, surface and margin covered with hair-like bristles. Inflorescence and fruit ripening occur in May-June. It is found in dry rocky, sloping slopes and deposits of lime in the low and moderate-high belts (Fig. 4).



Fig. 3. General view of *O. michauxii*



Fig. 4. General view of *O. buhseana.*

Table 2. Some environmental variables of *O. altissima, O. michauxii, O. buhseana*

Ecogeographical factors	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Altitude (m)	1206	2209	941	1433	1281	1360	1360	1590
Slope inclination	3%	8%	30%	30%	16%	30%	30%	30%
Slope orientation	W	SE	W	E	SE	S	S	W
Max. temp., °C	17.4	19.1	11.4	15.9	15.4	15.4	15.4	15.4
Min. temp., °C	9.83	7.2	3.56	4.3	4.6	4.6	4.6	4.6
Annualprecipitation(mm)	93.82	267	134.07	293	429	429	429	429
Biotope	reserve, roadside	roadside, meadow	roadside, forest stripe or forest side	roadside, meadow	rocky place	roadside,shrubbyplace	roadside,shrubbyplace	stony-gravelly slope
Collectionsite	100-1000 m^2	10-100 m^2	10-100 m^2	>1000 m^2	10-100 m^2	100-1000 m^2	100-1000 m^2	>1000 m^2
Relief	plain	hill	verticalslope	verti-calslope	foothill	verti-calslope	verti-calslope	verti-calslope
The mechanical composition of the soil	clay soil	mixed	stony-gravelly	mixed	stony-gravelly	sandy	sandy	stony-gravelly
Degreeofpasture	no	no	no	slightly	slightly	no	no	strong
Soilerosion	no	no	no	no	no	no	no	yes
Quantityratio	abundant enough (40-100)	scattered (100-150 sm)	scattered (100-150 sm)	scattered (100-150 sm)	scattered (100-150 sm)	abundant (20-40 sm)	abundant (20-40 sm)	scattered (100-150 sm)

In Azerbaijan, its distribution range is not large. According to the literary data, *O. buhseana* is found in Nakhchivan (Tamamshyan, 1954), Armenia (Tamamshyan, 1962), and Iran (Ranjbar, 2012). Herbarium specimens *O. buhseana* were not found. Herbarium specimens were collected from the village of Nimakesh in the Lerik district in 2019 (Figure 3).

Some environmental features of *O.altissima, O. Michauxii,* and *O. buhseana*: bioecological assessment of the ecological condition of wild sainfoins was conducted. For this purpose, environmental observations reflecting general information were carried out. The table of the data like altitude, sides, annual maximum and minimum temperature, annual precipitation, biotope, collection site, relief, the mechanical composition of the soil, degree of pasture, soil erosion, quantity ratio (Drude's scale) were prepared to analyze the environmental features (Table 2).

As can be seen from the table, *O. altissima*is found in Shamkir district (lowest alt. 941 m, min. tem. 3.56°C) and Nakhchivan AR (max.altitude 2209 m, max. tem. 19.1°C) where the least altitude and temperature difference was observed. Thus, the distribution range of this species is not only in the moderate-high belts but also in all high belts.

Although the low mountain belt forests are more exposed to anthropogenic influences, the spread of this species across all mountain belts allows it to expand its range. In addition, *O. altissima* is an arid and cold-tolerant crop and is found in areas with different environmental conditions. It is more resistant to climatic factors of the environment. Thus, species of *O. altissima* have a special agricultural significance and their involvement in breeding is advisable. *O. michauxii* and *O. buhseana* are found on stone-gravelly, sandy, not grazed, and non-eroded soils, mainly in steep slope areas.

REFERENCES

Asgarov A.M. (2016) The plant world of Azerbaijan, 258 p.

Grossheim A.A. (1952) Flora of Caucasus. **Vol. 5:** p. 369.

Hedge I.C. (1970) *Onobrychis* (*Leguminosae – Hedysareae*). In: P.H.Davis, D.F.Chamberlain,

V.A.Matthews (eds.).*Flora of Turkey and the East Aegean Islands*, Edinburgh, UK: Edinburgh University Press, **3:** 560-589.

Khinthibidze L.S. (1960) Monografii. Caucasian representatives of the genus *Onobrychis* Adans. Section of *Eubrychis* DC. Tiflis (Rus). **3:** 101-114.

Lock J.M. (2005) Tribe *Hedysareae*. In: G.Lewis, B.Schrire, B.Mackinder &M.Lock (eds.).*Legumes of the world*. Royal Botanical Gardens:p. 489–490

Miller P. (1754) Granadilla, Passion – flower, Gard. Dict. Abr. (ed. 4). [The volume has no page numbers] Available from:<http://www.tropicos.org/Reference/100000805> (accessed 23 January 2017).

Polhil R.M. (1981) *Hedysareae* DC. In: R.M.Polhill & P.H.Raven (eds.). *Advances in legume systematics*. Royal Botanical Gardens, **1:** 367–370

Ranjbar M., Hajmoradi F., Karamian R. (2012) An overview on cytogenetics of the genus *Onobrychis* (*Fabaceae*) with special reference to O. sect. *Hymenobrychis* from Iran. *Cytologia*, **65(3):**187-198.

Ranjbar M., Roya K., Anahita H. (2010) Cytosystematics of three *Onobrychis* species (*Fabaceae*) in Iran. *Caryologia*, **63(3):** 237-249; doi: 10.1080/00087114.2010.10589733.

Sirjaev G. (1925) *Onobrychis* generis revisio critica. Pars prima. *Publications Faculte des Sciences de l'Université Masaryk*, **56:** 58–68.

Tamamshyan S.G. (1962) *Onobrychis*. In: *Flora of Armenia*. Erevan: Academy of sciences of Armenistan SSR, **4:** 249–270 (in Russian).

Tamamshyan S.G. (1954) Flora of Azerbaijan. Baku: **5:** 458-470 (in Russian).

Yildiz B., Ciplak B., Aktoklu E. (1999) Fruit morphology of sections of the genus *Onobrychis* Miller (*Fabaceae*) and its phylogenetic implications, *Israel Journal of Plant Sciences*, **47(4):** 269-282.

Azərbaycan florasında bəzi xaşa növlərinin (*O. altıssıma, O. mıchauxıı* and *O. buhseana*) yeni yayılma sahələri və onların bioekoloji xüsusiyyətləri

Əzizxanlı Xarıbül Məmmədxan qızı, Əsgərov Aydın Musa oğlu

AMEA-nın Genetik Ehtiyatlar İnstitutu, Bakı, Azərbaycan

Məqalədə 3 xaşa növünün 8 yeni yayılma sahələri aşkar edilmiş, onların bioekoloji və fitosenoloji xüsusiyyətləri öyrənilmişdir. Yeni yayılma sahələri Kiçik Qafqazın şimal hissəsini, Talış və Naxçıvan bölgələrini əhatə edir. Məqsəd Azərbaycan florasında xaşa cinsi növlərinin yayılma areallarının dəqiqləşdirilməsi və seleksiya üçün quraqlığa, şaxtaya daha davamlı növlərin aşkar edilməsidir. Məqalədə toplanılan xaşa növlərinin yeni yayılma sahələrinə aid xəritə, ekoloji məlumatları əks etdirən cədvəl və foto şəkillər verilmişdir.

Açar sözlər: *Xaşa, populyasiya, bioekologiya, fəsilə, cins, növ, areal*

Новые ареалы распространения некоторых видов эспарцета *(O. altıssıma, O. mıchauxıı И O. buhseana)* во флоре Азербайджанаи их биологические особенности

Азизханлы Харыбюль Мамедхан кызы, Аскеров Айдын Муса оглу

Институт генетических ресурсов НАН Азербайджана, Баку, Азербайджан

Выявлено 8 новых ареалов 3-х видов эспарцета, изучены их биоэкологические и фитоценологические особенности. Новые ареалы распространения охватывают северную часть Малого Кавказа, Талышскую и Нахичеванскую области. Цель исследования заключалась в уточнении ареалов распространения эспарцета во флоре Азербайджана и выявлении видов, более устойчивых к засухе и морозам, для вовлечения их в селекционные работы. В статье представлены карта ареалов новых видов эспарцета, таблица, отражающая экологическую информацию, и фотографии.

Ключевые слова: *Эспарцет, популяция, биоэкология, семейство, род, вид, ареал*

Journal of Life Sciences & Biomedicine, vol. 4(77), No 1, p. 28-32 (2022)

The importance and role of sericulture development and silkworm breeding in the economy of Azerbaijan

***Bakirov Quduret Mammad, Shukurova Zarintaj Yusif, Mustafayev Agil Gulmammad**

Sheki Regional Scientific Center, Azerbaijan National Academy of Sciences, 24 L.Abdullayev Str., Sheki AZ 0055, Azerbaijan
****For correspondence**: quduret.bekirov@mail.ru*

Received: March 01, 2022; Received in revised form: March 11, 2022; Accepted: April 08, 2022

There are special importance and role of Sericulture development and Silkworm breeding in the economy of Azerbaijan. The development of this sector will resolve the unemployment problem in Azerbaijan and its regions, provide the residents with enough jobs, and will result in the promotion of the welfare level and financial situation of people that will significantly contribute to the solution of socio-economic problems in the region.

Keywords: *Silkworm, feeding, wet cocoon, dry cocoon, raw silk*

Sericulture is one of the most ancient fields of agriculture. Hundreds of years ago, the "Silk Road" had an important role in the development of commercial ties between the eastern and western countries in the world.

The famous historian, Adalat Tahirzada, in his work, "the Historical Sources of Shaki" stated that different regions of Azerbaijan, especially Shamakhi and Shaki, had been globally well known for their qualitative silk industry during ancient years. Shaki and Shamakhi have had a long history of establishing silk trading ties with Iran, Russia, Arabic, and European states for a long period of time (Tahirzada, 2005).

According to sericulture scientists A.K.Seyidov and B.H.Abbasov in their work, "Principles of Sericulture", the silk produced in Azerbaijan has been one of the main merchandises of the country during the XII-XIV centuries. The fame of this merchandise has been even promoted during XVI-XVII in Italy, French, Russia, Iran, Turkey, and other European and Asian countries (Saidov, 2012).

In his work, "The Methods of Improvement of Economic Incomes of Sericulture", R.Hajiyev stated that besides the determination of economic profits of sericulture in Azerbaijan, the factors effective in the promotion of the sector should also be identified and the local possibilities with a positive effect on these factors should be addressed as well (Hajiyev, 1966).

A.M.Qambarov in his book, "Sericulture and Silk Industry in Azerbaijan", has stated that the organizations dealing with sericulture and silk production should extensively apply more effective utilization of sericulture products (Qambarov, 1965).

In their research work, "the Study of Some Issues in Regard of Raw Material Economy in Azerbaijani Textile Industry", M.B.Taghiyev and Z.T.Mammadov state that the replacement of old machinery and equipment with the new ones not only will promote the preparations for cocoon unwinding and reeling processes but also will result in labor productivity increase by 50-60% and this will eventually lead to a decrease of productions costs by40% (Taghiyev, 1962).

The silkworm breeders have their unique role development of the Azerbaijani sericulture industry in course of the history and the industry played a significant role in the promotion of breeders' welfare in the said zone. The situation of Azerbaijan in the "Silk Road" route resulted in the formation and the development of the industry in Azerbaijan and caused the establishment and reinforcement of commercial ties with other countries. The breeding and selection science has also had a significant role

10.5281/zenodo.7239447
Available online 30 June 2022

in the development of sericulture and the realization of new records and achievements in the industry. Textile products including silk are one among the goods highly demanded by people in their day-to-day life. Taking into account the role of sericulture in the socio-economic life of people in the Azerbaijan economic zone in the USSR era and the fact that it has been the only large-scale sericulture industry point in the territory of the USSR, the first and the only silk production plant was established in this zone in Transcaucasia, which was the third one throughout the USSR territory from production capacity point of view; its products had been distinctive both for their quality and diversity. The institutions exported their products to all republics of the USSR and gained remarkable fame for their outstanding quality. The sericulture industry of Azerbaijan experienced a new boom in the 60s-70s years during which the annual wet cocoon production reached 7800 tons. The cocoon is a lightweight and the most expensive raw material in the textile industry and could only be produced in zones with favorable climatic conditions for production. The plants for processing cocoons and production of silk textile products are also selected for their climatic conditions (Saidov, 2012). Azerbaijan is a country with favorable conditions for sericulture and silk industry and the most suitable zone in the country for silkworm breeding and cocoon production is the northwest zone of the country. Such an expensive and effort-demanding industry should be employed for welfare and improvement of lifestyle in the community. The old fame of Azerbaijani silk should be regained once again, and it should take its place in global markets as deserved.

The small family and individual-based silk plants and processing facilities were being worked in Azerbaijan economic zone in the past with limited production capacity. The fame of such valuable products gradually reached foreign countries which caused higher demands from the marketplace. The sericulture in Azerbaijan economic zone experienced its utmost boom and hit the peak development in XVIII-XIX. The silk produced in the zone was mainly exported to Russia and then reexported to European states. The silk produced in Shaki had its unique place due to its quality and had been the most expensive one. Taking this into account the first silk plant was established in Nukha (Shaki) in 1829 and started to produce silk in 1830. The plant's production capacity was 555.2 tons by 1861 (Alizada, 2007). During 1913-1914 one million and five hundred people were directly engaging in the silkworm breeding industry in the Transcaucasia region out of which one million were living in the territory of Azerbaijan.

At that period, Azerbaijan started to export its products to Italy, Russia, and some other countries in the world. The businessmen for the time being started to invest in the industry and imported and installed silk processing equipment with high production capacity; they prepared new staff to deal with the industry and became courageous in buying the new products. As a result, thousands of people were employed in the industry in both urban and rural zones, the unemployment problem was eliminated, and the state economy boomed. Such a tradition continued during the USSR era and the largest silk plant in the Transcaucasia was established in Shaki. Taking into account the exceptional role of the sericulture industry in the development of the socio-economic and cultural life of the people in the Shaki-Zaqatala economic zone of Azerbaijan, the construction of the silk plant was started in 1926 which was the third one throughout the USSR for its production capacity which put into operation in 1931 under the name of "Shaki Silk Institute". The Institute exported its products with unique quality and diversity to all republics of the USSR.

The plant continued the ever-increasing trend and its production capacity reached 240 tons of raw silk, 18.0 million square meters of fabric, 70.0 tons of silk yarn, 45.0 million square meters of ready fabric and the number of its workers increased to 7500. The industry created a job for thousands of rural residents dealing in silkworm breeding and cocoon production. This life both in rural and urban zones due to the creation of extensive employment opportunities improved highly. The development of the sericulture industry caused a halt in unemployment in the zone and created a job for thousands of people living in this zone.

Just like any other industry, the most advanced technologies are successfully employed in the developed countries of the world in the sericulture industry which are favorable from the point of view of the economy (Detailed Glossary of Sericulture, 2013). The production of qualitative silk conforming to international standards is an issue of high importance. The governmental agencies pay

8.0 AZN for each kg of wet cocoon. The program has established a real ground for the development of sericulture in the country. To use in silkworm feed, the plantation of selected varieties of white mulberry trees has previously been started in favorable lands in Shaki-Zaqatala economic zone to provide feed for the ever-increasing silkworm breeding industry. The plants for high-quality silkworm eggs production should be established to provide the farmers with qualitative eggs parallel to the employment of the most advanced machinery and equipment which altogether with result in a boom of the industry and production of silks of higher quality.

The Republic of Azerbaijan will be able to achieve the annual production capacity of 5,000 MT of high-quality wet cocoon a year within a short 3-year period if all the above-mentioned issues are addressed properly through optimal solutions. Silkworm breeding is quite different from any other field of agriculture in terms of the production period; here the farmer completes the production chain within a short period of time and can supply the products to the market and earn money within a short time. The silkworm breeding and cocoon production is a field of agricultural profession, but the cocoon processing and silk production is an industrial process that needs the completion of several stages (killing the silkworm pupa, unwinding cocoon and production of raw silk and reeling to produce silk yarns, threading the yards, production of silk fabric, etc.). Consequently, in addition to the role of qualitative silkworm eggs, good feeding of the silkworm with white mulberry leaves, the silkworm breeding conditions, the degree of professionalism of silkworm breeders, and seasonal climatic conditions there are also other factors effective in the production of qualitative silk including the application of modern technology which altogether complete the chain of production of qualitative silk (Abbasov, 2009). Taking into account the above-mentioned factors, the suitable zone, i.e. Northwest zone of the country, was selected for the first state silkworm breeding and silk production industry. This will be the cornerstone for the production of qualitative silk production whereby we can lay down the proper ground that enables us to move to the next stage.

The next stage of the industry is the production of qualitative silk in conformity to the requirements laid down in the most recent international standards. In line with this stage, the majority of cocoon unwinding equipment currently installed in the silk workshops and plats should be replaced automated modern and advanced unwinding equipment should be imported, installed, and put into operation together with the mechanical ones. The works to be undertaken in the second stage should be aligned with the works undertaken for the first stage to continuously process the produced cocoon throughout the year and lay down the ground for the development of the industry in the future.

Based on Decree No. 2893, issued by the President of the Republic of Azerbaijan "on Promotion of Technology and Equipment of Sericulture in the Republic of Azerbaijan, issued on May 19th, 2017 and Decree No. 3286, issued by the President of the Republic of Azerbaijan regarding "State Program for Development of Silkworm Breeding and Sericulture in Azerbaijan" on October 6th, 2017, the required funds have been allocated to the establishment of the pedigree silkworm in Gakh, development of additional silkworm breeding sites and their equipment with modern equipment, and supply of breed mulberry seedlings as well as to pay as a gift to the people dealing with silkworm breeding.

The allocation of 5 AZN as subsidies for the production of each kg cocoon to the silkworm producers encouraged the farmers to shift to sericulture. The measures taken for the encouragement of farmers to sericulture have indeed been evaluated as important socioeconomic steps which will have a significant role in the increase of sericulture production, export of high-quality silk, creation of new jobs, and provision of rural people with the job which if fact.

From this point of view, we believe that the implementation of government programs dedicated to sericulture development will result in the sericulture and silkworm breeding boom and the auxiliary industries.

The high demand for silk in the global market resulted in an ever-increasing trend in the price of this merchandise. Presently the price of one MT of raw silk equals 20 MT of cotton wool and this resulted in a continuous increase in raw silk production. Although silk accounts for 0.2 percent of the global textile market share, silkworm breeding and sericulture have been developed in more than 30

countries throughout the world. Our country is capable to produce 8000 MT qualitative wet cocoon (equaling to 2960 dry cocoon) and by using such a qualitative raw material in the silk industry, we'll achieve the following results:

Based on the base price paid to each kg of a cocoon, 8 AZN, we can produce qualitative raw silk worth for 8.8 million AZN as well as use 540 MT in the second phase to produce medium-quality silk amounting to 640,000 AZN. In addition, the 200 MT cocoon not unwound in the first stage and an additional 200 MT suitable for unwinding will be effectively used in the processing which in turn will result in the creation of new jobs and capable to return 500,000 AZN additional income. In general, because of the implementation of the second phase of the sericulture development plan in the country, we annually will gain 9-10 million AZN additional income and the conditions will be prepared for the employment of 4500-5000 people.

The third stage of the sericulture development plan includes the production of reeled silk from raw silk, threaded yarn, production of silk fabrics, processing the silk fabrics, dyeing silk fabrics, their screen and press printing, and preparation of qualitative silk fabrics in different designs. If 450 MT qualitative raw silk produced by cocoon unwinding equipment is totally allocated to the production of natural silk fabric of high quality, 3.5-4.0 million square meter fabrics will be produced. The remaining 160 MT silk of medium quality could be used for the production of silk kerchiefs, yard for sewing, and production of silk carpets. Consequently, around 2500-300 people will be employed in this stage in the production of silk kerchiefs, silk yarn and silk carpets.

In the fourth and last stages of the sericulture development plan, we can produce silk carpets from threaded yarns and create jobs for 800 to 1000 people and produce a lot of valuable products.

The breeding of more productive breeds of silkworm will play a paramount role in the sericulture industry in our country. Just like any other sector dealing with the production of goods for supply to the market, the free market prevailing in our country's economy puts forward its requirements for the sericulture sector. Presently, sericulture is applying advanced and more economic methodology and processes in many countries using the most advanced technologies and achievements. In line with such a trend, about 60 kg of silkworm eggs were imported and distributed between the farmers which include 2070 boxes of silkworm eggs. 2 or 3 workers are required to breed a box of silkworm eggs up to cocoon production. As a result, 4000-6000 people will be employed for breeding 2070 boxes of silkworm eggs. The created jobs will even be more in the coming years due to an increase in the number of distributed egg boxes. The creation of such productive jobs will halt the unemployment problem in the country, especially in the northwest region. In this way, if the people engage in the sericulture field permanently, new jobs will be created, the unemployment problem will be resolved and their welfare and financial situation will be promoted consequently will not seek any job abroad and avoid the problems they normally face in the foreign countries. 370 kg of silkworm seeds were imported to our republic and distributed to the population in 2017. This means 12760 boxes of silkworms. To feed 12760 boxes of silkworms, to get the cocoon harvest – and this is to provide employment of 25520-38280 workforce. In such cases the population will provide with jobs in rural and urban areas of Azerbaijan, the unemployment rate and the welfare of the population will improve day by day. As a result of these works, it was laying up 244 tons of cocoon in 2017 in our republic.

We suggest that the proper solutions should be found for the following issues all bearing scientific and paramount importance. Addressing these problems through proper solutions will play a drastic and significant role in the elimination of the unemployment problem in rural districts of the Republic of Azerbaijan.

The development of sericulture and silkworm breeding in Azerbaijan especially in the Shaki-Zagatala economic zone can play an important role in the elimination of the unemployment problem in this zone. The development of this sector will resolve the unemployment problem in the region, provide the residents with jobs and will result in the promotion of welfare level and financial situation of the people which will significantly contribute to the solution of socio-economic problems in the zone.

The resistant and qualitative silkworm strains and hybrids bred by us have high importance from an economic point of view. The use of silkworm

breeds and hybrids prepared as a result of research conducted by the "Mulberry and Silkworm Breeding Branch" of Shaki Regional Scientific Center, affiliated with the Azerbaijan National Academy of Sciences is economically and scientifically justifiable and capable to promote the productivity level of the industry and improve the economy and socio-economic situation of the people in the zone.

REFERENCES

Abbasov B.H. (2009) The theoretical and practical principles of breeding in sericulture. Ganja: 274 p.

Alizada Z., Ibrahimov N. (2007) Shaki silk in long Caravan Roads. *Master Publications,* Baku: 244 p.

Chiraqzada V.A. (1988) The old sericulture land. Baku: 158 p.

Detailed Glossary of Sericulture (2013) Baku: Apostroff, 356 p.

Hajiyev R.Sh. (1966) Methods of increasing the economic incomes of sericulture. Baku: Azernashr, 41 p.

Qambarov A.M. (1965) Sericulture and silk industries in Azerbaijan. Baku: Azerbaijan State Publications. 94 p.

Seydov A.K., Abbasov B.H. (2012) Principles of sericulture. Baku: 164 p.

Taghiyev M.B., Mammadov Z.T. (1962) The study of some issues in regard of raw material economy in Azerbaijani textile industry. *Economics Institute affiliated with USS Azerbaijan Academy of Science,* **XII:** 62.

Tahirzada A. (2005) The historical sources of Shaki. *Master Publications,* Baku: 250 p.

İpəkçiliyin və baramaçılığın inkişafının Azərbaycan iqtisadiyyatında əhəmiyyəti və rolu

Bəkirov Qudurət Məmməd oğlu, Şükürova Zərintac Yusif qızı, Mustafayev Aqil Gülməmməd oğlu

AMEA-nın Şəki Regional Elmi Mərkəzi, Şəki, Azərbaycan

İpəkçiliyin və ipəkçiliyin inkişafının Azərbaycan iqtisadiyyatında xüsusi əhəmiyyəti və rolu vardır. Bu sahənin inkişafı Azərbaycanda və onun regionlarında işsizlik problemini həll edəcək, sakinləri kifayət qədər iş yerləri ilə təmin edəcək və əhalinin rifah səviyyəsinin və maddi vəziyyətinin yüksəldilməsi ilə nəticələnəcək ki, bu da sosial problemlərin həllinə mühüm töhfə verəcək. -zonada iqtisadi problemlər.

Açar sözlər: *İpəkqurdu, yemləmə, yaş barama, quru barama, xam ipək*

Роль и значимость развития шелководства и производства коконов щелкопряда в экономике Азербайджана

Бекиров Гудурат Мамед оглу, Шукюрова Заринтадж Юсиф кызы, Мустафаев Агиль Гюлмамед оглу

Шекинский региональный научный центр НАН Азербайджана, Шеки, Азербайджан

Развитие шелководства и коконоводства в Азербайджане приобретает особое значение в экономике республики. Расширение этой сферы в регионах Азербайджана приведет к устранению безработицы, обеспечит занятость населения, улучшит материальное благосостояние и, таким образом, сыграет важную роль в решении социально-экономических проблем.

Ключевые слова: *Червь тутового шелкопряда, кормление, сырой кокон, сухой кокон, шелк сырец*

Journal of Life Sciences & Biomedicine, vol. 4(77), No 1, p. 33-39 (2022)

Cross-scoring of cardiovascular and somatometric ratios at normal and elevated values in schoolchildren (15–17 years)

Panahova Gultakin Ibad

Academician Abdulla Garayev Institute of Physiology, Azerbaijan National Academy of Sciences, 78 A.M. Sharifzadeh Str., Baku AZ1100, Azerbaijan
For correspondence: *gpanahova82@gmail.com*

Received: March 09, 2022; Reviewed: April 08, 2021; Accepted: May 25, 2022

The work was carried out on the basis of the Azerbaijan Medical University and secondary schools in Baku. Correlation of blood pressure (BP), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), myocardial indicator dispersion (MI), heart rhythm indicator (RI) with somatometric indexes - body mass index (BMI) in schoolchildren aged 15-17 are investigated; a combined change in blood pressure (BP) with a myocardial indicator (MI) is shown: Percentile tables were used. It has been established that the increased values of these indicators are mainly formed in overweight groups with selective distribution of the skin-fat layer (SFL) in certain areas of the body. Cross-correlation analysis of hemodynamic parameters (SBP, DBP, MI) with somatometric (BMI, SFL) revealed the presence of selective functional relationships between them and their increase with an increase in the values of the BMI and the SFL. Complex shifts of the analyzed indicators indicate the formation of cardiometabolic risk in schoolchildren.

Keywords: *Blood pressure (BP), myocardial indicator (MI), body mass index (BMI), skin-fat layer (SFL), correlation*

ABBREVIATIONS

MI – myocardial indicator
BP - blood pressure
SBP - systolic blood pressure
DBP - diastolic blood pressure
HR - heart rate
IR - rhythm index
BMI - body mass index
SFL - skin-fat layer
Elevat. – elevation

INTRODUCTION

In accordance with the theory of systemogenesis of functional systems (Anokhin, 1968; Sudakov, 2012) and principles of heterochronous development of the organism (Butrova, 2001; Dudnik et al., 2005; Sudakov, 2012) in the formation of functional systems, a growing organism, unequal growth rate of different systems and body structures of children and adolescents leads to their disproportionate development, reflected in the formation of neuroendocrine and neurochemical regulatory mechanisms (Babenko et al., 2019; Dedov, 2004). All this disrupts vital physiological processes, including various indicators of the cardiovascular system: blood pressure, blood supply to the heart, which are aggravated by overweight, adynamia, emotional stress, etc. (Arshavsky, 1982; Dedov, 2004; Beisbekova et al., 2017; Netrebenko, 2017; Suder, 2017; Babenko et al., 2019).

According to WHO, more than 200 million school-age children and adolescents in the world currently suffer from overweight, accompanied by a violation of the indicators of the cardiovascular system (BO3, 2012, 2018; WHO, 2018).

In this regard, comprehensive studies of various indicators, blood pressure (BP) and indicators of the myocardium (MI) were conducted for the first time in this work, which allows judging early dispersion disorders of myocardial blood supply

10.5281/zenodo.7239481
Available online 30 June 2022

that is, about "transient" ischemia on the background of somatometric measurements, within normal and overweight schoolchildren (15-17 years old) (Mammedov et al., 2015, 2017).

The main goal of this research is the study of cardiovascular and somatometric ratios at normal and elevated values in schoolchildren (15-17 years old).

MATERIALS AND METHODS

This investigation was carried out on the basis of the Azerbaijan Medical University and secondary schools in Baku. The research was conducted with a contingent of schoolchildren aged 15-17, of both sexes. Investigated such indicators of the functional state of the cardiovascular system as SBP, DBP (mm Hg), and HR (bpm). The level of blood pressure with a set of cuffs for children measured by N.S.Korotkov's tonometer was considered elevated if SBP or DBP was equal to or exceeded the value of the 90th percentile of the scale of distribution of indicators for a given age, gender, and height. Heart rate was assessed by counting the pulsation of the radial artery for 1 minute.

The myocardial indicator was also researched for changes in the dispersion of low-amplitude ECG oscillations reflecting early manifestations of "transient" myocardial ischemia. Wherein, if the dispersion index of the myocardial indicator was within MI≤14%, then it was evaluated as a norm, and at elevated values of MI> 14%, it testified to the formation of "transient" myocardial ischemia (Mammedov et al., 2017). At the same time, the rhythm indicator (IR) was calculated, which is due to the presence of arrhythmia on the ECG, indicating the emotional stress of the body. Dispersion assessment of MI and IR was carried out by a non-invasive method, according to ECG, on the analyzer "Cardiovisor - 06C".

Somatometric, anthropometric measurements were carried out according to the generally accepted methodology: the body length, and then the body weight were determined (Babenko et al., 2019; Beisbekova et al., 2017; Dudnik et al., 2005). The size of the skin-fat layer (SFL) was also recorded in various parts of the body (shoulder blades, abdomen, forearm, thigh).

The measurements were carried out non-invasively, with an electronic digital Caliper (KEC-100) with a measurement discreteness of 1 mm (Babenko et al., 2019; Gerasimchuk and Girsh, 2019).

The determination of the nutritional status was based on the study of the body mass index - BMI (kg/m^2).

The results obtained in the studies were subjected to statistical processing using variational (U-Mann-Whitney) and correlation (Rho-Spearman) methods of analysis on a statistical package SPSS-22 (Arbackle, 2012; Gapanovich-Kaidalov et al., 2020).

RESULTS AND DISCUSSION

Investigated indicators of blood pressure and myocardial indicator in the age group of 15-17-year-old schoolchildren. In general, it was found that out of the analyzed 125 boys, it was revealed that 31 (24.8%) had high blood pressure (BP). With a normal body mass index, an increase in blood pressure was noted in 14.4% of cases, i.e. out of 104 people, 15. With elevated values of the body mass index, elevated blood pressure occurs in 79.2%, i.e. 16 out of 21 people. As regards the 128 girls we researched, 39 of them in 30.5% of cases, at normal body mass index values, and elevated blood pressure was found in 23.8% of cases (out of 101 people in 24). At elevated values of the body mass index, increased blood pressure was registered in 55.56% of cases or in 15 out of 27 people.

In this way, among schoolchildren aged 15-17 years of both sexes, compared to the normal values of this indicator elevated blood pressure values were mainly registered with elevated body mass index values.

In addition, based on the dispersion analysis of low-amplitude ECG oscillations, by means of a "Cardiovisor", the myocardial indicator (MI) and the heart rhythm index (IR) were also determined (Fig., A, B).

Depending on the quantitative values of the dispersion indicators of the myocardial indicator (MI) in 15-17-year-old schoolchildren the portrait of the heart in figure 1 (A, B) shows the "color" changes: A is the variance of IM within ≤14% and green color prevails, which indicates a normal blood supply (while blood pressure is normal); B - dispersion of MI>14%, the green color decreased, and gray, yellow, and red increased, which indicates the formation of "transient" ischemia (with

increased blood pressure).In this case, as well, these changes were accompanied by an increase in the values of IR ≥40% (In normal IR≥20)%.

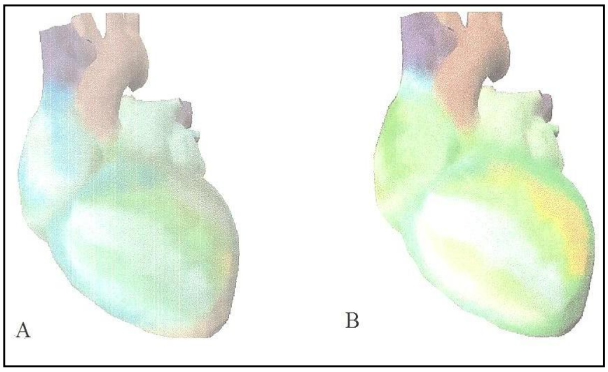


Fig. Comparison of heart portraits in children aged 15-17 in terms of MI.

At the same time, in our previous works, it was shown that (Mammadov et al., 2017) the increased values of MI(>14%) among boys with elevated blood pressure and BMI is 86.7% out of 31 people in 26, compared with 21.3% of cases (out of 94 people in 20) with increased MI, identified in the group with normal blood pressure values, as well as BMI (Table 1). Among 15-17-year-old schoolchildren of both sexes, statistically significant, combined and unidirectional changes in the values of MI with AT were revealed (in 80.7% of cases), mainly with elevated values (BMI, BP, MI).

As can be seen from Table 1, 15-17-year-old boys with overweight and elevated values of BMI, BP and MI, compared with the group with normal values, the indicators of IR, SBP, DBP and heart rate were respectively higher, specifically: 41.3%, 148.7+12.31 mm Hg, 97.7 mm Hg±3.17и 87.2 bpm±2.75. In girls, these changes were observed in the same percentage ratios and were higher in magnitude compared to normal indicators. Gender differences are shown in more detail in Table 1.

It is important to note that (Table 1) elevated values of BMI, BP and MI in boys, are accompanied by a selective increase in the values of the skin-fat layer (SFL) distributed over various parts of the body (shoulder blades-21.2±1.97, forearm-12.9±0.67, with the highest values of fat folds in the abdomen (26.8±1.88) and hip (28.8±2.52). In girls, due to gender characteristics, these indicators increase, respectively, reaching 24.6±1.92 and 31.8±1.31, and the adipose tissue in the area of the shoulder blades up to 26.9±2.38 they exceeding similar values in boys. The question arises whether are there and what are the functional relationships between hemodynamic and somatometric parameters?

Table 1. Values of SBP, DBP, heart rate, IR, as well as with SFL (shoulder blades, abdomen, forearm and hip) with normal and elevated BP, MI and BMI in schoolchildren aged 15-17 years of both sexes

BMI / Indicators	Normal body mass index (BMI)				Elevated body mass index (BMI)			
Gender differences	**Boys**		**Girls**		**Boys**		**Girls**	
BP, mm Hg	norm.	elevated	norm.	elevated	norm.	Elevated	norm.	elevated
Number of examined	n=89	n=15	n=77	n=24	n=5	n=16	n=12	n=15
MI, %	norm.	elevated	norm.	elevated	norm.	Elevated	norm.	elevated
Number of examined	n=72	n=12	n=59	n=19	n=2	n=14	n=5	n=13
Compatibility BP with MI, %	80.9	80.0	76.6	79.2	40.0	87.5	41.7	86.7
RI,%	≤26.2	≤27.0	≤26.4	≤28.1	≤35.7	≤41.3	≤36.3	≤44.7
SBP, mm. Hg	114.6±1.72	141.1±3.17***	110.7±1.03	137.5±1.03	116.2±2.64	148.7±2.31***	112.3±1.76	139.5±1.33
DBP, mm Hg	73.4±2.63	93.3±1.77***	71.7±1.22	81.6±2.23	78.1±2.36	97.7±3.16	68.6±0.94	85.8±2.67
HR, bpm	68.9±0.72	76.8±1.93***	74.9±1.01	86.8±2.11	70.8±1.54	87.2±2.75	69.7±2.04	84.2±2.58
SFL (mm) Shoulder blades	13.1±0.27	13.5±1.28	12.8±0.46	18.7±1.12	15.2±1.86	21.2±1.97*	22.3±1.17	26.9±2.38
Abdomen	13.5±0.34	24.4±1.56***	18.2±0.49	25.1±1.52***	19.1±1.47	26.8±1.88*	24.1±1.53	24.6±1.92
Forearm	5.4±0.28	8.3±0.66***	9.9±0.40	10.0±0.78	7.1±1.15	12.9±0.67***	12.2±0.87	12.4±1.18
Hip	16.9±0.42	19.1±1.84	22.1±0.67	27.0±1.47**	19.9±1.98	28.8±2.52*	31.1±2.49	31.8±1.31

Note: there are significant differences between indicators with normal or elevated BP and MI: *-p<0.05; **-p<0.01; ***-p<0.001.

Table 2. Correlation coefficients (R) of indicators SBP, DBP, MI and RI in relation to somatometric indicators (height, weight, BMI), and also SFL (shoulder blades, abdomen, forearm and hip), with normal and elevated values of BMI, BP and MI in 15-17 year-olds of both sexes.

Boys

			Indicators BMI, BP and MI, norma, n=72				Indicators BMI, BP and MI elevated, n=14			
			SBP	DBP	RI	MI	SBP	DBP	RI	MI
Spearman's rho	Height sm	R	0.180	0.166	0.044	0.112	0.278	0.201	-0.087	0.270
		Sig.	0.067	0.093	0.658	0.257	0.223	0.381	0.707	0.237
	Weight kq	R	0.514**	0.519**	0.485**	0.407**	0.569**	0.479*	0.091	0.431
		Sig.	0.000	0.000	0.000	0.000	0.007	0.028	0.696	0.051
	BMI Kq/sm^2	R	0.471**	0.468**	0.503**	0.348**	0.891**	0.705**	0.285	0.517*
		Sig.	0.000	0.000	0.000	0.000	0.000	0.000	0.210	0.016
	SFL: shoulder blades	R	0.511**	0.523**	0.518**	0.364**	0.712**	0.582**	0.314	0.546*
		Sig.	0.000	0.000	0.000	0.000	0.000	0.006	0.166	0.011
	Abdom.	R	0.534**	0.576**	0.506**	0.469**	0.620**	0.507*	0.164	0.480*
		Sig.	0.000	0.000	0.000	0.000	0.003	0.019	0.477	0.028
	Forearm	R	0.550**	0.558**	0.597**	0.376**	0.588**	0.481*	0.075	0.525*
		Sig.	0.000	0.000	0.000	0.000	0.005	0.027	0.747	0.015
	Hip	R	0.359**	0.400**	0.483**	0.545**	0.461*	0.338	-0.118	0.306
		Sig.	0.000	0.000	0.000	0.000	0.035	0.134	0.611	0.178

Girls

			Indicators BMI, BP and MI, norma, n=103				Indicators BMI, BP and MI elevated, n=14			
			SBP	DBP	RI	MI	SBP	DBP	RI	MI
Spearman's rho	Height sm	R	0.302**	0.293**	0.187	0.223*	0.240	0.216	0.010	0.162
		Sig.	0.002	0.003	0.061	0.025	0.227	0.279	0.962	0.419
	Weight kq	R	0.517**	0.493**	0.470**	0.409**	0.703**	0.656**	0.402*	0.582**
		Sig.	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.001
	BMI Kq/sm^2	R	0.545**	0.516**	0.534**	0.453**	0.867**	0.821**	0.570**	0.759**
		Sig.	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000
	SFL: Should. blades	R	0.530**	0.508**	0.409**	0.563**	0.605**	0.563**	0.662**	0.695**
		Sig.	0.000	0.000	0.000	0.000	0.001	0.002	0.000	0.000
	Abdom.	R	0.463**	0.445**	0.426**	0.535**	0.562**	0.505**	0.712**	0.635**
		Sig.	0.000	0.000	0.000	0.000	0.002	0.007	0.000	0.000
	Forearm	R	0.557**	0.546**	0.544**	0.568**	0.399*	0.321	0.539**	0.500**
		Sig.	0.000	0.000	0.000	0.000	0.039	0.103	0.004	0.008
	Hip	R	0.363**	0.324**	0.397**	0.462**	0.453*	0.348	0.603**	0.507**
		Sig.	0.000	0.001	0.000	0.000	0.018	0.075	0.001	0.007

Note: R - correlation coefficient by Spearman.
Sig. - statistical significance of the correlation coefficient: * - $p<0.05$; ** - $p<0.01$;

The results of the cross-correlation analysis showed, how functional connections between the analyzed structures change with changes in weight and redistribution of adipose tissue in different parts of the body (Table 2).

Increases in cross-correlation coefficients, (R) between hemodynamic parameters (SBP, DBP, MI) and weight indicators, BMI, and also distribution of the thickness of the skin-fat layer (SFL) in various parts of the body (shoulder blades, abdomen, forearm, and thigh)against the background of increased values of the latter, indicate an increased influence of somatometric indicators on the increase in blood pressure(BP) and"transient" ischemia is assessed by the myocardial indicator (MI). At the same time, in girls, the increase in cross-correlation coefficients is more significant than in boys.

All this may be related to the gender characteristics of girls in this period of development. It was further revealed that cross-correlation relationships of weight values, BMI and SFL with hemodynamic parameters (SBP, DBP, MI), increased and were higher than the links with height and IR indicators, which also confirms the greater dependence of increases in blood pressure and MI on the increase in body weight and the distribution of the

skin-fat layer in it. It is known that these changes stimulate an increase in the level of cortisol and catecholamines, which in turn leads to an increase in BP and MI (Dedov, 2004; Babenko et al., 2019).

Summarizing the results obtained in groups of 15-17-year-old schoolchildren of both sexes, we note that, symbiosis of elevated comorbid parameters and features of changes having common pathogenetic bases, evidence of the formation of cardiometabolic risk in schoolchildren during the period of growth and development (Butrova, 2001; Bekasbekova et al., 2017; Babenko et al., 2019).

CONCLUSIONS

1. A high combination of unidirectional changes in BP values with MI values was revealed.
2. Overweight and high BMI, hemodynamic parameters of BP, (SBP, DBP) and IR, MI were elevated compared with the group with normal weight and BMI. At the same time, the result also depended on the selective redistribution of the thickness of the SFL in individual parts of the body.
3. The increase in cross-correlation coefficients of the functional relationship between hemodynamic and somatometric parameters is accompanied by increases in BP and MI. All of this testifies to the complex effect of somatometric indicators on increases in blood pressure and ischemic manifestations of the myocardium.
4. In accordance with the theory of the functional systems of the body, the process of teaching schoolchildren aged 15-17 is accompanied by an increased “physiological price”, considered a cardiometabolic risk in achieving the final beneficial result.

REFERENCES

Анохин П.К. (1968) Биология и нейрофизиология условного рефлекса. М.: Медицина, 574 с.

Аршавский И.А. (1982) Физиологические механизмы и закономерности индивидуального развития. Москва: 270 с.

Бабенко А.Ю., Матвеев Г.А., Алексеенко Т.И., Деревицкий И.В., Кокина М.А., Шляхто Е.В. (2019) Взаимосвязи компонентов метаболического синдрома суровнем гормонов, вовлеченных в регуляцию метаболизма жировой ткани. *Артериальная гипертензия*, **25(6):** 639-652.

Беисбекова А.К., Айтенова А.Н., Датхабаева Г.К. (2017) Избыточная масса тела и ожирение у детей: причины, последствия, профилактика. *Вестник КазНМУ*, **№1:** 178-180.

Бутрова С.А. (2001) Метаболический синдром: патогенез, клиника, диагностика, подходы к лечению, Русский медицинский журнал.,**9(2):** 56-60.

ВОЗ (2012) Ожирение и избыточный вес. *Информационный бюллетень* **№311.** [Электронный ресурс]. Режим доступа: http://www.who.int/mediacentre/factsheets/fs311/ru/index. - дата доступа 20.08.2019.

Гапанович-Кайдалов Н.В., Шаршакова Т.М. (2020) Медицинская статистика и основы доказательной медицины: учеб. метод. пособие для студентов 4–6 курсов всех факультетов и магистрантов учреждений высшего медицинского образования. Гомель: ГомГМУ, 44 с.

Герасимчук О.А., Гирш Я.В. (2019) Композиционный состав тела у детей и подростков с ожирением. *Трансляционная медицина*, **6(1):** 51-57.

Дедов И.И. (2004) Влияния висцеральной жировой ткани на гормонально-метаболические показатели при абдоминальном ожирении и артериальной гипертонии. В кн.: *Ожирение и метаболизм*, Москва: **часть 1:** 26-29.

Дудник Е.Н., Глазачев О.С., Судаков К.В., Бокерие Л.А., Рева М.П. и др. (2005) Возможности использования ЭКГ анализатора «Кардиовизор-06С» в функциональной диагностике и прогнозировании ишемии миокарда. *Функциональная диагностика* (Москва), **№1:** 8-14.

Мамедов А.М., Алиев А.Х., Панахова Г.И. (2015) Системные исследования у подростков пубертатного периода, корреляционных взаимоотношений соматометрических характеристик с физиологическими и дисперсионными ЭКГ показателями. *Матер. XXI всемирного конгресса по реабилитации в медицине и иммунореабилитации*. Сингапур // *Аллергология и иммунология*, **6:** 120-123.

Мамедов А.М., Алиев А.Х., Панахова Г.И.,

Джафарова А.М. (2017) Комплексные исследования артериального давления, индикатора миокарда, индекса массы тела и толщины кожно-жирового слоя у 11-14 летних школьников обоего поле. *Естественные и технические наука* (Москва), **№9:** 56-61.

Нетребенко О.К., Украинцев С.Е., Мельникова И.Ю. (2017) Ожирение у детей: новые концепции и направления профилактики. Обзор литературы. *Вопросы современной педиатрии,* **16(5):** 399–405.

Судаков К.В. (2012) Теория системогенеза. М.: Горизонт, 2012, 565 с

Arbackle J. (2012) IBM SPSS Amos 21: Uzer's Guide. IBM Corp.: Amos Development Corporation, 680 p.; http:/www.twirpx.com/ file/933135.

Mammedov A.M., Aliyev A.Kh., Panahova G.I., Jafarova A.M. (2017) Study on relations among increase in blood pressure values, dispersions of the myocardial indicator, body mass index and thickness of the skin – fat layer in 15-17. *South Asian J. Exp. Biol.,* **7(2):** 69-73.

WHO (2018) Situation of child and adolescent health in Europe. WHO: 231 p.

Məktəblilərin (15-17 yaş) normal və yüksəlmiş ürək-damar və somatometrik göstəricilərinin krosskorrelyasiya əlaqələrinin tədqiqi

Pənahova Gültəkin İbad qızı

AMEA-nın Akademik Abdulla Qarayev adına Fiziologiya İnstitutu, Bakı, Azərbaycan

İş Azərbaycan Tibb Universitetinin və Bakı şəhər orta təhsil məktəblərinin bazasında görülüb. Tədqiqatlar, 15-17 yaşlarında olan məktəblilərin sistolik arterial təzyiqi (SAT), diastolic arterial təzyiqi (DAT), miokard indikatorun (Mİ) dispersiyası və ürəyin ritm indeksi (Rİ) göstəriciləri ilə somatometrik göstəriciləri (bədən kütləsi-BK, bədən kütləsi indeksi-BKİ, Dəri Piy Qatının –DPQ qalınlığı) əlaqələrin tədqiqi aparılmışdır. Müəyyən olunmuşdur ki, BKİ normal olan 104 nəfərdən, yalnız 15-də, (14.4%) arterial təzyiqin (AT) yüksəlməsi qeydə alınmışdır. Lakin BKİ yüksək olan 21 nəfərdən isə, 16-da (79.2%) AT yüksək olmuşdur və risk qrupunu təşkil etmişdir. Ilk dəfə olaraq arterial təzyiqlə (AT) və Mİ dispersiyasının eyni vaxtda birgə tədqiqi aparılmışdır. Göstərilmişdir ki, bu göstəricilərin dəyişikliyi bir biri ilə əlaqəli və eyni istiqamətli olmuşdur. Müəyyən olunmuşdur ki, tədqiq olunan ürək-damar sistemi göstəricilərin yüksəlməsi əsasən artıq bədən kütləsi olan qruplarda və onların DPQ qalınlığının bədənin ayrı-ayrı nahiyyələrində müxtəlif olaraq paylanması ilə əlaqədardır. Kardiogöstəricilər və somatometrik göstəriciləri ilə aparılan krosskorrelyasiya təhlili əsasında aşkarlanmışdır ki, onların arasında seçici funksional əlaqələri mövcuddur və bu isə somatometrik göstəricilərin kəmiyyətcə artması ilə əlaqəlidır. Beləliklə, somatometrik komponentlərin krosskorrelyasiya təsirinin artması kardiogöstəricilərin yüksəlməsinə gətirib çıxarır. Təhlil olunan kompleks göstəricilərin artması, kardiometabolik riskin formalaşmasını göstərir və həmin qrup məktəblilərdə tədris prosesinin yüksək fizioloji dəyər hesabına başa gəlməsini göstərir.

Açar sözlər: *Arterial təzyiq (AT), miokard indikatoru (MI), bədən kütləsi indeksi (BKI), dərialti piy qatı (DPQ), korrelyasiya*

Кросскорреляционные исследования сердечно-сосудистых и соматометрических соотношений при нормальных и повышенных их значениях у школьников (15-17 лет)

Панахова Гюлтакин Ибад кызы

Институт физиологии имени академика Абдуллы Гараева НАН Азербайджана, Баку, Азербайджан

Работа выполнена на базах Азербайджанского Медицинского Университета и средних школ г. Баку. Исследованы взаимоотношения систолического артериального давления (САД), диастолического артериального давления (ДАД), дисперсий индикатора миокарда (ИМ), индикатора ритма (ИР) сердца с соматометрическими показателями, с индексом массы тела (ИМТ) и распределением кожно-жирового слоя (КЖС) на отдельных частях тела у школьников 15-17 лет. Выявлено, что при нормальном ИМТ, повышение АД было отмечено в 14.4% случаев, т.е. у 15 из 104 человек. При повышенных значениях ИМТ, АД увеличивается в 79.2% случаях, т.е. у 16 из 21 человек этот показатель был выше нормы и составлял группу риска. Впервые проведены одновременные исследования артериального давления (АД) с дисперсией ИМ. Показаны сочетанные, однонаправленные изменения этих показателей. Установлено, что повышенные значения указанных показателей, в основном формируются в группах с избыточном весом и в связи с избирательным распределением величин кожно-жирового слоя (КЖС) на отдельных участках тела. Кросскорреляционный анализ, кардиопоказателей (САД, ДАД, ИМ) с соматометрическими (ИМТ, КЖС) показателями выявил наличие между ними избирательных функциональных связей и их увеличение при повышении значений ИМТ, КЖС. Таким образом, эти данные свидетельствуют о возрастании кросскорреляционного влияния соматометрических компонентов на формирование повышенных значений кардиопоказателей. Комплексные сдвиги анализируемых показателей и их увеличение указывают на формирование кардиометаболического риска, рассматриваемого, как повышение «физиологической цены» учебного процесса у школьников.

Ключевые слова: *Артериальное давление (АД), индикатор миокарда (ИМ), индекс массы тела (ИМТ), кожно-жировой слой (КЖС), корреляция*

Journal of Life Sciences & Biomedicine, vol. 4(77), No 1, p. 40-45 (2022)

Study of the molecular mass of AZ-130 biomolecule and its stability at low pH

[1]*Aghayeva Aytan Gabil, [2] Streatfield Stephen John, [1] Huseynova Irada Mammad

[1]Institute of Molecular Biology & Biotechnologies, Azerbaijan National Academy of Sciences, 11 Izzat Nabiyev, Baku AZ1073, Azerbaijan;
[2]Department of Biotechnology, Fraunhofer USA Mid-Atlantic Center, Newark DE 19711, United States of America
***For correspondence:** aytanaghayeva@gmail.com*

Received: March 07, 2022; Reviewed: April 29, 2021; Accepted: May 26, 2022

The aim of this work was to determine the approximate value of the molecular mass of an exogenous biomolecule synthesized by the *Bacillus vallismortis* AZ-130 strain isolated from oil-contaminated soils of Azerbaijan and to determine the degree of its stability at low pH values. To achieve the goals, strain AZ-130 was cultivated for 24 hours on a shaker with constant shaking (180 rpm) at 32°C. Next, a supernatant was obtained from the culture solution, which, after the addition of trifluoroacetic acid and 10-fold concentration using 3K MWCO Amicon Ultra-15 Centrifugal Filter Devices centrifugal filter concentrators, was examined by the growth inhibition assay for the presence of activity against *S. aureus* ATCC 29213. Based on the obtained results, it was found that the bioactive molecule produced by the AZ-130 strain is stable at low pH values and has a molecular mass of more than 3000 Daltons.

Keywords: *Antimicrobial activity, antibiotics, bioactive molecules, natural products, stability, pathogenic bacteria*

INTRODUCTION

Antibiotic-resistant bacteria have been on the rise in recent years, and unfortunately almost all commercially available antibiotics are slowly losing their effectiveness. This becomes a serious health problem (Ventola, 2015; Prestinaci et al.,2015). Of particular concern are six clinically pathogenic bacteria that Louis Rice summarized under the abbreviation "ESKAPE" (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and Enterobacter spp.) (Rice, 2008). For bacterial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Acinetobacter baumannii* (CRAB), vancomycin-resistant *E. coli* (VRE), and multidrug-resistant (MDR) *Pseudomonas aeruginosa* (MDR) for treatment fewer antibiotics are available than 10 years ago (van Duin and Paterson, 2016; Minuta and Arias, 2016; Cetinkaya et al., 2000). The discovery of penicillin by Alexander Fleming in 1928 made a significant contribution to the treatment of infections caused by microorganisms (Fleming, 1944).

According to Newman and Cragg, 70% of antibacterial drugs on the market from 1981 to 2019 are natural products or their derivatives, 28% - synthetic drugs, 1% - imitation of natural products and pharmacophores (Schneider, 2021). Natural products and their structure play a significant role in the development of antibiotics (Smith, 2000; Harvey, 2008). Microorganisms are the most potential source for the production of natural antibacterial drugs (Wright, 2014). The number of bacteria that can theoretically be examined for the presence of new secondary metabolites is enormous. On the other hand, it is estimated that approximately 98% of microorganism species have not been identified to this day and are not subject to cultivation in the laboratory (Wade, 2002; Pham and Kim, 2012). Therefore, it is necessary to develop new, safe and effective antimicrobial compounds to fight pathogens. A recent success in the field of antibiotics was the discovery of

10.5281/zenodo.7239523
Available online 30 June 2022

teixobactin, a novel antibiotic that was isolated using an iChip isolation chip approach in 2015 (Ling et al, 2015; Nichols et al., 2010). Cultivation strategies that aim to isolate bacteria from unexplored and unanalyzed sources allow the development of new natural products with completely new structures and biological activities (Challinor and Bode, 2015; Pidot et al, 2014).

The process of developing new drugs is very expensive and time-consuming (Ekins et al., 2019; Wright, 2018). In most cases, in the last stages of the development process, the bioactive molecule is eliminated due to the rediscovery of an already known compound (Atanasov et al., 2021). That is why it is very important to include in the development process the steps of dereplication of bioactive compounds (Schneider, 2021; Carrano and Marinelli, 2015). One of the tools for dereplication is to determine the molecular weight of a bioactive compound in the early stages of development. Identification of the producer strain at the molecular level and information about the approximate molecular weight of the compound of interest will help reduce the likelihood of rediscovery of a known molecule. Moreover, the process of discovering the antimicrobial compound from natural products includes several purification steps of the compound of interest from the cell culture supernatant. It's well known that the purification of antimicrobials involves the use of different buffers with different pH values. That's why, before any small- or large-scale purification, it is necessary to ensure that the compound of interest is stable at low pH.

Bacillus vallismortis strain AZ-130 could be a candidate that produces a novel antimicrobial compound (Агаева, 2019; Aghayeva et al., 2021). The goals of this study are to determine the degree of AZ-130 biomolecule stability at low pH and its approximate molecular mass.

MATERIALS AND METHODS

The object of study was AZ-130 antibacterial compound synthesized by the *Bacillus vallismortis* strain AZ-130 isolated from an oil-contaminated soil sample of Azerbaijan in 2014.

To determine the degree of stability of biomolecule at low pH, 100 ml of TB medium was inoculated with 1 ml of a bacterial suspension at OD_{600} = 0.5–0.6 and incubated at 180 rpm and 32°C for 24 h. After a 24-hour incubation, the culture was centrifuged at 10000 g for 15 min at 4° C and the supernatant was purified from the cell culture by filtration through a 0.22 µm PES membrane. Next, trifluoroacetic acid (TFA, a final concentration of TFA - 0.1%) was added to 1 ml of the supernatant, gently shaken, and centrifuged again at 10,000 g for 15 min at 4°C, followed by filtration through a 0.22 µm PES membrane. The resulting material was analyzed for the antibacterial activity against *S. aureus* ATCC 29213 by the growth inhibition assay. The screening was performed by the soft-agar overlay method as described by Hockett (Hockett and Baltrus, 2017; Balouiri et al., 2016) with some modifications. For screening, 10 µl of material was plated onto an agar plate confluent with the indicator strain - *Staphylococcus aureus* ATCC 29213. The plates were left to dry for 5 minutes under a hood and incubated at 37°C for 24 hours. The range of antibacterial activity (zone of inhibition (ZOI)) was expressed in millimeters as the diameter of the transparent zone (the zone where the growth of the test organism was suppressed). As a control, all the above steps were repeated with TB media.

To determine the approximate molecular mass of the bioactive molecule, the producer strain was inoculated into 50 ml of TSB medium and cultivated overnight at 32°C and 180 rpm. The next day, culture supernatants were clarified from the cell culture by centrifugation at 10000 g for 15 minutes at 4°C, then by filtration through a 0.22-µm polyethersulfone (PES) membrane. A 100 µl sample was taken from the collected supernatant for activity analysis and stored at 4°C. 15 ml of the supernatant was concentrated 10-fold (to 1.5 ml) using 3K MWCO Amicon Ultra-15 Centrifugal Filter Devices centrifugal filter concentrators. The concentration time was 67 minutes; the volume of the concentrate (retentate) is 1.5 ml. The pooled supernatant, retentate, and filtrate at various dilutions (undiluted (x), 2x diluted (x/2), 4x diluted (x/4), and 8x diluted (x/8)) were applied to the Petri dishes with TSA medium, confluent with soft agar containing the indicator strain and incubated for 20–24 h at 37°C. ZOI were measured.

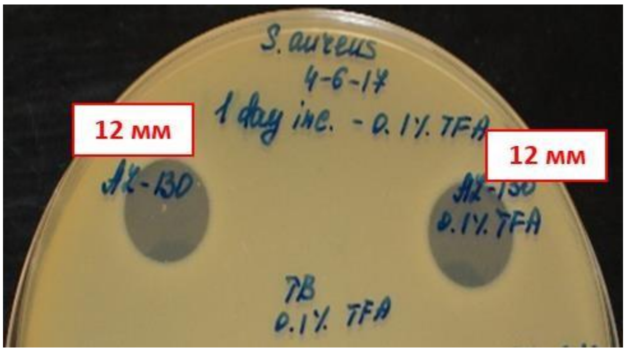


Fig. 1. Stability analysis of the supernatant of AZ-130 strain at low pH.

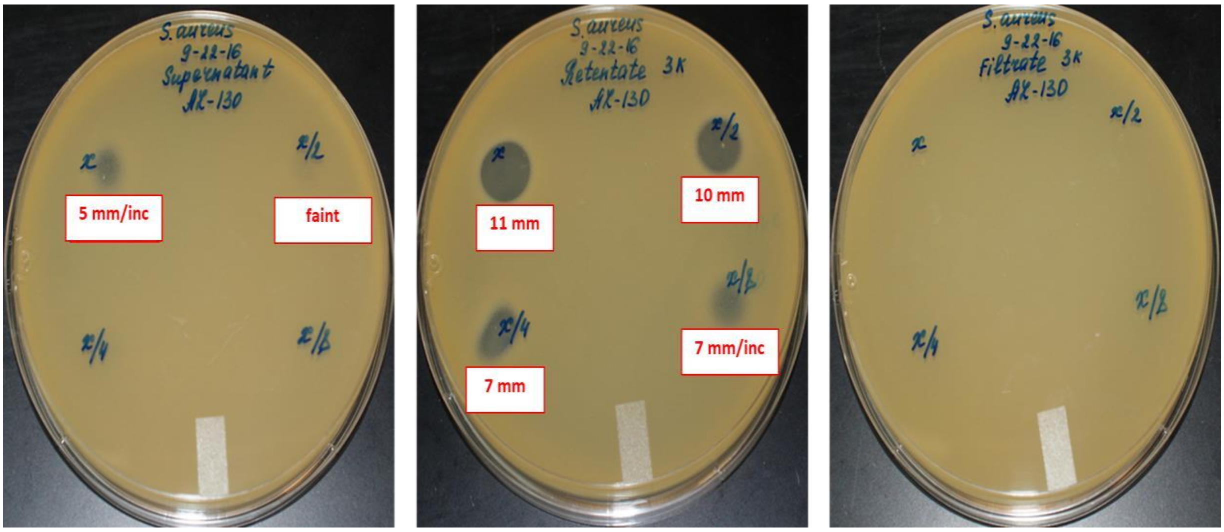


Fig. 2. The results of the analysis of the supernatant, retentate and filtrate in various dilutions against *S. aureus* ATCC 29213.

RESULTS AND DISCUSSION

For the characterization and purification of AZ-130 biomolecule, it was necessary to elucidate the degree of its stability at low pH and determine the approximate molecular mass. Figure 1 shows the results of the analysis of the AZ-130 supernatant at low pH. As can be seen from the figure, the activity of AZ-130 does not change before and after the addition of 0.1% TFA, being 12 mm in both and indicating the resistance of AZ-130 biomolecule to low pH. There was no activity in the controls (TB and TB+0.1% TFA).

Determination of the approximate molecular mass of AZ-130 biomolecule was carried out using 3K MWCO Amicon Ultra-15 Centrifugal Filter Devices centrifugal filter concentrators. As it can be seen in Figure 2, the activity of the undiluted supernatant, equal to 5 mm/partial, after 10-fold concentration increases to 11 mm. In addition, the 4-fold and 8-fold diluted supernatant had no activity, while the 4-fold and 8-fold diluted concentrate showed 7 mm and 7 mm/incomplete activity, respectively. There was no activity in the filtrate.

CONCLUSIONS

AZ-130 strain showed strong activity against gram-positive opportunistic *pathogenic S. aureus* and *E. faecalis* strains (Агаева, 2019) during initial and supernatant screenings. By 16S rRNA gene sequencing AZ-130 strain was identified as *Bacillus vallismortis*. It was found that strain AZ-

130 produced a single compound with antibacterial activity with the retention time at HPLC column 12.854 min (Aghayeva et al., 2021).

The AZ-130 bioactive molecule is stable at low pH values. This is very important for the correct choice of appropriate solutions in the processes of further purification of selected biomolecules from the cell supernatant. Moreover, the results of 10-fold concentration of the AZ-130 supernatant using Amicon Ultra-15 Centrifugal Filter Devices 3K MWCO centrifugal filter concentrators showed that the molecular mass of the AZ-130 bioactive compound is greater than 3000 Da. A search in the APD3 database revealed that there are currently no known antibiotics produced by *B. vallismortis* bacteria with molecular mass equal to or bigger than 3000 Da.

ACKNOWLEDGEMENT

This article was prepared based on the results of experiments carried out during the 2014-2018 years at the Fraunhofer USA Center for Molecular Biotechnology (currently Fraunhofer USA, Mid-Atlantic Center, Department of Biotechnology). The presented work is a part of the doctoral thesis to be submitted to the Institute of Molecular Biology & Biotechnologies of Azerbaijan NAS.

REFERENCES

Агаева А.Г. (2019) Создание бактериальной коллекции на основе штаммов, изолированных из почв Азербайджана и их скрининг на наличие новых антибактериальных биомолекул. *Журнал Наук о Жизни и Биомедицины*, **1(74), № 2:** 13-19.

Aghayeva A.G., Streatfield S.J., Huseynova I.M. (2021) AZ-130 Strain from Oil-contaminated Soil of Azerbaijan: Isolation, Antibacterial screening, and optimization of cultivation conditions. *Microbiology*, **90(6):** 754–762.

Atanasov A.G., Zotchev S.B., Dirsch V.M., The International Natural Product Sciences Taskforce & Claudiu T.S. (2021) Natural products in drug discovery: advances and opportunities. *Nature Reviews Drug Discovery*, **20:** 200–216.

Balouiri M., Sadiki M., Ibnsouda S.K. (2016) Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm. Anal.*, **6(2):** 71-79.

Carrano L., Marinelli F. (2015) The relevance of chemical dereplication in microbial natural product screening. *Journal of Applied Bioanalysis*, **1(2):** 55-67.

Cetinkaya Y., Falk P., Mayhall C.G. (2000). Vancomycin-resistant enterococci. *Clin. Microbiol. Rev.*, **13(4):** 686–707.

Challinor V. L. & Bode H. B. (2015) Bioactive natural products from novel microbial sources. *Ann. NY Acad. Sci.*, **1354:** 82–97.

Ekins S., Puhl A.C., Zorn K.M., Lane T.R., Russo D.P., Klein J.J, Hickey A.J., Clark A.M. (2019) Exploiting machine learning for end-to-end drug discovery and development. *Nature Materials*, **18:** 435–441.

Fleming A. (1944). The discovery of penicillin. *Br. Med. Bull.*, **2:** 4–5.

Harvey A.L., Edrada-Ebel R., Quinn R.J. (2015) The re-emergence of natural products for drug discovery in the genomics era. *Nat. Rev. Drug Discov.*, **14:** 111–129.

Hockett K.L., Baltrus D.A. (2017) Use of the soft-agar overlay technique to screen for bacterially produced inhibitory compounds. *J. Vis. Exp.*, **119:** 55064.

Ling L.L., Schneider T., Peoples A.J., Spoering A.L., Engels I., Conlon B.P., Mueller A., Schäberle T.F., Hughes D.E., Epstein S. et al. (2015) A new antibiotic kills pathogen without detectable resistance. *Nature*, **517:** 455-459.

Munita J.M., Arias C.A. (2016) Mechanisms of Antibiotic Resistance. *Microbiol Spectr.*, **4(2)**; doi: 10.1128/microbiolspec.VMBF-0016-2015.

Nichols D. et al. (2010) Use of ichip for high-throughput in situ cultivation of ‘uncultivable’ microbial species. *Appl. Environ. Microbiol*., **76:** 2445–2450.

Pham V.H.T., Kim J. (2012) Cultivation of unculturable soil bacteria. *Trends Biotechnol.*, **30:** 475–484.

Pidot S.J., Coyne S., Kloss F., Hertweck C. (2014) Antibiotics from neglected bacterial sources. *Int. J. Med. Microbiol.*, **304:** 14–22.

Prestinaci F., Pezzotti P., Pantosti A. (2015) Antimicrobial resistance: a global multifaceted phenomenon. *Pathog. Glob. Health.*, **109(7):** 309-318.

Rice L.B. (2008) Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. *J. Infect. Dis.*, **197:** 1079.

Schneider Y.K. (2021) Bacterial natural product drug discovery for new antibiotics: Strategies for tackling the problem of antibiotic resistance by efficient bioprospecting. *Antibiotics*, **10(7):** 842.

Smith T. (2000) Antibiotics from soil bacteria. *Nat Struct Biol.*, **7(3):** 189-190.

van Duin D., Paterson D.L. (2016) Multidrug resistant bacteria in the community: Trends and lessons learned. *Infect. Dis. Clin. North. Am.*, **30(2):** 377–390, 420.

Ventola C.L. (2015) The antibiotic resistance crisis. **Part 1:** Causes and threats. *Pharmacy and Therapeutics*, **40(4):** 277–283.

Wade W. (2002) Unculturable bacteria - the uncharacterized organisms that cause oral infections. *J. R. Soc. Med.*, **95(2):** 81–83.

Wright G.D. (2014) Something old, something new: revisiting natural products in antibiotic drug discovery. *Can. J. Microbiol.*, **60:** 147–154.

Wright G.D. (2018) Unlocking the potential of natural products in drug discovery. *Microbial Biotechnology*, **12:** 55–57.

AZ-130 biomolekulunun molekul kütləsinin və pH-ın aşağı qiymətində sabitliyinin tədqiqi

[1] Ağayeva Aytən Qabil qızı, [2] Streatfield Stephen John, [1] Hüseynova İradə Məmməd qızı

[1]AMEA-nın Molekulyar Biologiya və Biotexnologiyalar İnstitutu, Bakı, Azərbaycan
[2] Orta Atlantika Mərkəzinin Biotexnologiya Departamenti, Fraunhofer, ABŞ

Təqdim olunan işin məqsədi Azərbaycanın neftlə çirklənmiş torpaqlarından ayrılmış *Bacillus vallismortis* AZ-130 ştamının sintez etdiyi ekzogen biomelokulun aşağı pH-da stabillik dərəcəsinin və molekul kütləsinin təxmini qiymətinin müəyyən edilməsi olmuşdur. Qarşıya qoyulmuş məqsədlərə nail olmaq üçün AZ-130 ştamı 24 saat ərzində 180 rpm və 32°C-də kultivasiya edilmişdir. Daha sonra kultural məhluldan alınan supernatantın aktivliyi trifluoroasetik turşu əlavə edildikdən və 3K MWCO Amicon Ultra-15 Centrifugal Filter Devices istifadə edərək 10 dəfə qatılaşdırıldıqdan sonra böyümənin inhibə edilməsi metodu ilə *S. aureus* ATCC 29213 qarşı tədqiq edilmişdir. Əldə edilmiş nəticələrə əsasən AZ-130 ştamı tərəfindən istehsal olunan bioaktiv molekulunun pH-ın aşağı qiymətlərində stabil olması və onun molekul kütləsinin 3000 Daltondan çox olması müəyyən edilmişdir.

Açar sözlər: *Antimikrob aktivlik, antibiotiklər, bioaktiv molekullar, təbii məhsullar, sabitlik, patogen bakteriyalar*

Изучение молекулярной массы биомолекулы AZ-130 и ее стабильности при низком значении уровня pH

[1] Агаева Айтан Габиль кызы, [2] Стритфилд Стивен Джон, [1] Гусейнова Ирада Мамед кызы

[1]Институт молекулярной биологии и биотехнологий НАН Азербайджана, Баку, Азербайджан
[2] Центр Средней Атлантики, Фраунхофер, США

Целью представленной работы было установление приблизительного значения молекулярной массы экзогенной биомолекулы, синтезируемой выделенным из нефтезагрязненных почв Азербайджана штаммом *Bacillus vallismortis* AZ-130 и определение степени ее стабильности при низких

значениях pH. Для достижения поставленных целей штамм AZ-130 в течение 24 часов культивировали на шейкере при постоянном встряхивании (180 об/мин) и температуре 32°C. Далее из культурального раствора был получен супернатант, который после добавления трифторуксусной кислоты и 10-кратного концентрирования с использованием центробежных фильтрующих концентраторов 3K MWCO Amicon Ultra-15 Centrifugal Filter Devices, был исследован методом подавления роста на наличие активности против *S. aureus* ATCC 29213. На основе полученных результатов установлено, что биоактивная молекула, продуцируемая штаммом AZ-130, стабильна при низких значениях pH и имеет молекулярную массу более 3000 Дальтон.

Ключевые слова: *Антимикробная активность, антибиотики, биоактивные молекулы, натуральные продукты, стабильность, патогенные бактерии*

Journal of Life Sciences & Biomedicine, vol. 4(77), No 1, p. 46-52 (2022)

Chemical components of leaf essential oil of *Vitex agnus-castus* L. in the Absheron conditions

***Mammadova Irada Oruc, Mammadova Zumrud Aman, Alakbarli Ramiz Alakbar**

Institute of Dendrology, Azerbaijan National Academy of Sciences, Mardakan Settl., 89 Yesenin Str., Baku AZ1044, Azerbaijan
****For correspondence:*** *irade_mamedov@mail.ru*

Received: January 26, 2022; Received in revised form: March 01, 2022; Accepted: May 20, 2022

Medicinal plants are used all over the world because of their lower risk of side effects and environmentally friendly, cost-effective production compared to chemical drugs, encouraging researchers to do more research. One of the most popular medicinal plants is *Vitex agnus-castus* L., grown in tropical and subtropical regions, with various health benefits. We have studied the chemical composition of the leaf essential oils of *Vitex agnus-castus* L. introduced to Absheron. Essential oils from fresh leaves of *Vitex agnus-castus* L. were obtained by hydrodistillation and their volatile components were identified by GC/MS. Leaves of *Vitex*, grown at the Institute of Dendrology, have a light yellow liquid with an essential oil concentration of 0.45%. More than 20 components have been identified in the essential oil of the leaves. The leaf oil also contained large amounts of 1,8-cineole (28.31%) and β-pinene (13.1%). The results are given by comparing studies conducted on the same species in our country and in the world.

Keywords: *Vitex agnus-castus* L.*, leaves, essential oil, component composition*

INTRODUCTION

Due to its natural and climatic conditions, Azerbaijan is similar to many regions of the Mediterranean - the main world center for the cultivation of essential oils and medicinal plants. Types and forms of aromatic plants are a source of important groups of biologically active substances, such as essential oils, which have a high content of essential components. Essential oils are used in many industries, including pharmacology, perfumery, and cosmetology. As the properties of essential oils are studied, their range of application expands, and the demand for essential oils and aromatic substances increases year by year. Sacred Vitex (*Vitex agnus-castus* L.) is one of these essential oil plants that have a special place in Azerbaijan. Vitex is a promising tree-like plant with a spherical open crown, 2-4 m tall.

The leaves are large and complex, consisting of 5-7 leaves. Numerous flowers collected in narrow, but large and dense paniculate spike-shaped inflorescences, which appear in large numbers at the ends of almost all shoots. The crown of the flower has two lips, lavender, lilac, or pale purple. This plant blooms from June to October. The plant's odor is very strong, but at the same time, it is pleasant. They are mainly pollinated by insects. The fruits are dry four-celled spherical nuclei, 3-4 mm in diameter, with dark blue flowers, covered with bark, and ripen in October-November.

The literature shows that the chemical components of *Vitex* leaves have terpenes and flavonoids. Their biologically active substances extensively studied in previous years. Our goal is to study the composition of the essential oil of the leaves of this plant grown in Absheron, to apply it in our future research, and expand its use in the Republic of Azerbaijan.

MATERIALS AND METHODS

The research was conducted in 2021 in the laboratory of Essential Oils of Plants at Mardakan

10.5281/zenodo.7239571
Available online 30 June 2022

Institute of Dendrology (Absheron region), in accordance with the pharmacopoeia method. Existing technologies for the processing of essential oil raw materials ensure its preparation. Various methods are used for this: grinding, casting, drying, fermentation, sonication, and infrared radiation. To obtain the essential oil, the leaves of the Institute's collection were used (Fig. 2).



Fig. 1. General view of the plant *V. agnus-castus*

(a)



(b)



Fig. 2. a - flowers and b - fruits of *V. agnus-castus.*

Extraction of essential oil in the laboratory was carried out by hydro-distillation in the Klevenzer apparatus (Jennings, 1980; Tyagi, 2017). Raw material cut into 1-3 cm pieces was used as a sample. Collection of the studied material - calamus leaves was carried out in July during the flowering stage of the plant, in the middle-aged generative state of ontogeny (4th year of life) in natural populations. The essential oil is obtained by hydro-steam distillation for at least 10 hours until the release of the essential oil is stopped. The duration of the hydro-steam distillation process was determined experimentally based on the study of the dynamics of changes in the productivity of essential oils over time. For analysis, we used hexane solutions of the plant's essential oil (1.0-3.0% by weight). The mass fraction of the main components of the essential oil was determined by gas chromatography on a chromatograph "Chromatec-Crystal 2000 M" using the pole column. Capillary column CR is 5 cm, length 30 m, inner diameter 0.25 mm. Phase - 5% phenyl 95% polysylphenylene siloxane, film thickness - 0.25 µm. The thermostat temperature is programmed from 4°C / min to 750°C to 240°C. Evaporator temperature - 250°C. Carrier gas - helium, flow rate 1 ml / min. The temperature of the transition line is 250°C; the temperature of the ion source is 200°C. Electron ionization - 70 eV. The scanning range is 20-450. Scan time - 0.2. The sample volume of the essential oil is 0.2 µL. The normalization method was used to calculate the mass fraction of the components (Tkachev 2008; Adams, 2007). The results of experimental research were developed using mathematical-statistical methods.

The following formula was used for this:

$$\boldsymbol{X} = \frac{\boldsymbol{V} \times \mathbf{100}}{\boldsymbol{m}}$$

V - volume of oil obtained in mm

M - grams of raw material used

The component composition of the separated essential oils was analyzed by chromatography on the gas chromatograph KRISTAL-2000M (Russia), using the computer program Crystal PM-1.

The composition of the components was calculated from the peak areas and the identification of the individual components was based on a comparison of the retention times and total mass spectra with the relevant data for the components of the reference oils and pure compounds (Boelens, 1991). Data from the Wiley 275 library of mass

spectra (275,000 mass spectra) (Hussein, 2014) and atlases of mass spectra and linear capture indices (Shchipitsina, 2010) were also used for identification. Identification was considered final with the complete coincidence of mass spectra and linear capture indices (Chryssavgi, 2008; Ayvaz, 2010).

RESULTS AND DISCUSSION

The essential oil of the common twig is a light yellow liquid. Twenty components were identified in the essential oil of the common twig, of which 19 components were found in quantities exceeding 0.2% and accounting for 80.6% of the whole oil (Table 1). Analysis of the component composition of the essential oil of the sacred Vitex raw material, removed during the flowering phase of plants, showed that its main components are terpene hydrocarbons: α-pinene, sabinene, β-pinene, monocyclic monoterpenes, bicyclic monoterpenes, monoterpene alcohols, monoterpene ketones, and oxides; 1,8-cineole.

Table 1. The main components of *V. agnus-castus* essential oil are obtained under the conditions of Absheron

№	Retention time	Components	Mass fraction of the component, %
1	8.694	α-pinene	6.1
2	9.35	limonene	9.4
3	10.46	1,8-cineole	28.3
4	14.16	linalool	2.8
5	9.46	β-pinene	13.1
6	9.031	sabinene	0.14
7	9.71	carene-3	1.8
8	10.27	α-terpinene	2.6
9	11.10	γ-terpinene	0.5
10	12.94	terpinolene	0.3
11	15.15	terpinene 4-ol	1.04
12	14.61	caryophyllene	0.2
13	15.32	citronellol	2.95
14	15.68	N,N di methyl acetamide	3.84
15	16.21	estragole	2.82
16	16.29	camphene	4.95
17	17.15	camphor	0.4
18	17.25	citronellyl butyrate	0.14
19	19.96	citronellyl tiglate	0.3
20	20.80	geranyl tiglate	5.4

The chemical composition of *Vitex agnus-castus* L. EO and the chromatogram are presented in table 1 and Fig. 3 (Ulukanli, 2015). The oil yield was 1.3% (v/w) based on fresh weight. The main component was 1,8-sineole (28.3%). The combination of bicyclic and monoterpene alcohols (34.81%) had the highest percentage among other constituents, this combination included, α-pinene (6.09%), β-pinene (13.1%), geranyl tiglate (5.36%) and camphene (4.95%).

V. agnus-castus essential oil contains several tens of mono- and sesquiterpenoids. α-pinen and β-pinene, 1,8-cineole, linalool, camphene, geranial tiglate, and terpinen-4-ol are present in the greatest amount (Tkachev, 2008; Senatore, 1996).

The qualitative and quantitative compositions of essential oil have been studied in sacred Vitex growing in Nigeria (Hamid, 2010), Amazonia (Zoghbi, 1999), Italy (Galletti, 1996), Turkey (Hüseyin, 2014), Brazil (Zoghbi, 1999), Morocco (El-Kamari, 2018), Southern coast of Crimea (Bogatyuk, 2015) and in other countries. In addition, the composition of the volatile components of the essential oil is significantly influenced by its genetic, geographical origin, and growing conditions (Chryssavgi, 2008; Boelens, 1991). According to the literature, the main producers are Turkey, Italy, and Brazil (Hüseyin, 2014; Adams, 2007).

The main components of *V. agnus-castus* essential oil from Nigeria are β-pinene (20.0%), viridiflorol (9.8%), α-pinene (9.1%), 1,8-cineole (6.7%), β-farnesene (5.4%), terpinen-4-ol (4.2%), α-terpineol (4.1%), and β-pellandrene (4.1%). The essential oil was extracted from the leaves of *V. agnus-castus* in southern Italy, and the highest component in the leaves was 1,8-cineole (35.2%), sabinene (23.6 %), α-pinene (7.6%); studied the volatile components of the leaves of the *V. agnus-castus* plant growing in the Amazon region. Components with the highest content: in the leaves; β-farnesene (5.2%), 1.8 cineole (33.5%) and sabinene (18.5%); the components of the leaves of the plant of the sacred Vitex native to Brazil were studied, and the highest component in the leaves was 1,8-cineole (23.8%), β-farnesene (14.6%), caryophyllene (12.5%), sabinene (11, 4%), α-terpinyl acetate (7.7%), etc. The highest concentration in the essential oil of *V. agnus-castus* is 1,8-cineole (35.2%), sabinene (23.6%), α-pinene (19.48%).

The essential oil content in Vitex leaves ranges from 0.02% (Hina Zahid Ghazala, 2016) to 1.3% (Katiraee, 2015) (Table 3).

When growing within the Mediterranean region, this figure in plants ranges from 0.2 to 0.8%; outside the natural range (Brazil, Nigeria) is also quite high - 0.3 and 0.8%, respectively. The content of the main component of 1,8-cineole also varies widely - from 6.7 to 50.9%.

Analysis of the available data on the mass fraction of the main components (Table 2) suggests a significant variability in the component composition of Vitex essential oil in different ecological and geographical conditions.

A comparison of the components of Vitex oil shows that the quality and quantity of oil are very different. Thus, even inside the dominant components: sabinene and pinene (predominance of monoterpenes), caryophyllene and farnesene (predominance of sesquiterpenes) (Table 2) the chemical components of Vitex essential oils also differ, the mass fraction of 1,8-cineole is completely absent, the clear predominance of α-pinene and β-pinene allows this oil to be delivered to the pinene component (Hamid, 2010; Zoghbi, 1999; Katiraee, 2015).

Table 2. Qualitative and quantitative compositions of the essential oil of *V. agnus-castus* leaves according to the literature data

Component	Nigeria	Turkey	Crimea	Brazil	Iran	Pakistan	Morocco	Italy
α-pinene	9.1%	26.9%	4.78%	8.9%	19.4%	9.0	9.76%	4.0%
sabinene	1.2%	22.7%	6.8%	18.5%	6.89%	10.8%	14.57%	-
β-pinene	20.0%	1.3%	-	-	-	-	-	-
1,8-sineole	6.7%	14.20%	19.1%	33.5%	-	50.9%	19.61%	15.6%
viridofloral	9.8%	-	15.9%	-	-	-	-	-
β-farnesene	5.4%	8.5%	15.8%	5.2%	-	-	-	8.6%
cisocymene	8.4%	-	-	-	-	-	-	-
transcarifilene	-	9.13%	14.3%	-	-	-	-	-
α-terpinyl acetate	-	-	-	6.4%	-	-	-	-
bicyclogermarkene	-	2.4%	13.7%	3.2%	-	2.4%	-	-
β-caryophyllene	1.1%	8.9%	12.8%	2.8%	8.5%	6.5%	9.5%	8.9%
β-caryophyllene oxide	-	0.6%	-	0.8%	-	-	5.0%	0.5%

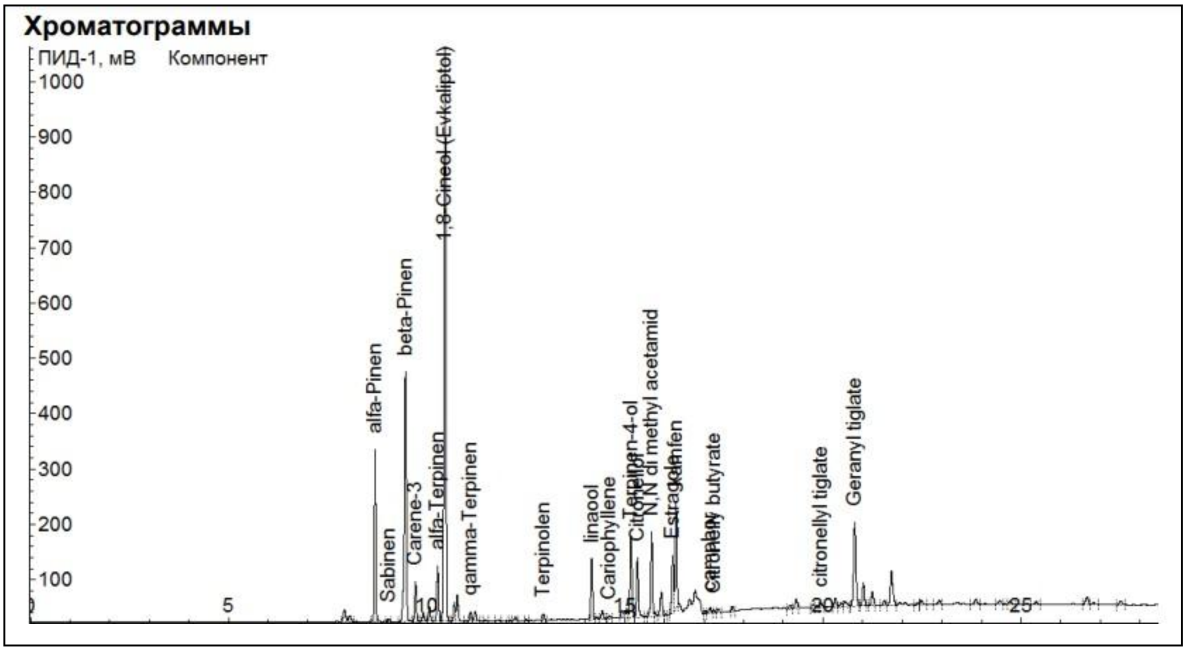


Fig. 3. Chromatogram of *V. agnus-castus* essential oil on SR-5 column.

Table 3. Characteristics of the essential oil of the *V. agnus-castus* leaves from various cultivating areas

№	Cultivation place	Mass fraction of the essential oil for absolutely dry mass, %	Mass fraction of 1,8- cineole, %
1	Pakistan	0.02	50.9
2	Turkey	0.20	24.3
3	Brazil	0.3	33.5
4	Morocco	0.35	8.7
5	Southern coast of Crimea	0.79±0.1	21.8
6	Iran	1.3	13.3
7	Nigeria	0.8	6.7

Most of the studied essential oils can be attributed to 1,8-cineole. This is mainly typical for plants growing in the Mediterranean countries, as well as under cultivation conditions in Brazil (Zoghbi, 1999) and Pakistan (Hina Zahid Ghazala, 2016).

The main features of the sacred Vitex essential oil, which is grown or cultivated in different places, are the predominance of 1,8-cineole or pinene, as well as the presence of an inverse relationship between the group of monoterpenes (sabinene and pinene) and sesquiterpenes (farnesene, caryophylline).

From such changes in the composition of essential oils, Vitex seems to depend on the reserve's climate and growing conditions (El-Kamari, 2018).

CONCLUSIONS

According to research, the main components of the essential oil of the *Vitex agnus-castus* L. during flowering are 1,8-cineol and β-pinene. The components mentioned in the literature were also key components in our study. Considering the natural bioactive compounds that have therapeutic potential, *V. agnus-castus* L. is one of the best-selling and most widely used medicinal plants in the world.

In general, studies of the components of the essential oil of Vitex (although the proportions of the components are different) have shown that they have a similar distribution.

This shows us that the essential oils of *V. agnus-castus* do not differ much even in different geographical regions. It shows that although the plant has a different location, its main components do not change drastically; it was noted that only the coefficients differ.

However, *in vivo* and *in vitro* studies and more clinical experiments are needed to study the composition of the beneficial compounds of this plant and the associated mechanisms of influence.

REFERENCES

Məmmədov T.S. (2010) Abşeronun ağac və kol bitkiləri. Bakı: Elm və təhsil, 127 s.

Məmmədov T.S. (2019) Azərbaycanın dendroflorası. Bakı: Elm, **V cild,** 370 s.

Каррыев М.О. (1973) Фармакохимия некоторых эфиромасличных растений флоры Туркменистана. Ашхабад: Илим, p. 22-27.

Ткачев А.В. (2008) Изучение летучих веществ растений. Новосибирск: 969 с.

Шипичина О.С., Ефремов А.А. (2010) Компонентный состав эфирного масла различных вегетативных частей дягиля лекарственного Сибирского региона. *Химия раст. сырья*, **4:** 115-119.

Adams R.P. (2007) Identification of essential oil compounds by gas chromatography/quadrupole mass spectroscopy. USA: Allured Pub. Corp., 804 p.

Ataslar E. (2004) *Saponaria kotschyi* Boiss üzerinde morfolojik ve anatomik araştırmalar. (*Caryophyllaceae*). *Turk. J. Bot*., **28:** 193-199.

Ayvaz A., Sagdic O., Karaborklu S. (2010) Insecticidal activity of the essential oils from different plants against three stored-product insects. *Journal of Insect Science*, **10:** 21.

Blamey M., Gray-Wilson C. (1998) Mediterranean Wild Flowers. London: HarperCollins Publisher, UK, 560 p.

Boelens M.H., Jimenez R. (1991) Chemical Composition of the essential oil from the gum and various parts of *Pistacia lentiscus* L. (mastic gum tree). *Flavor and Fragrance Journal*, **6:** 271-275.

Boqatyuk N.P., Danilova I.L., Pexova O.A. (2015) Dinamika nakopleniya i sostav efirnoqo masla vitexsa svyaşennoqo (*Vitex agnus-castus* L.) v predqornoy zone Krıma. *Ximiya i bioloqiya. Elektronnıy naucnıy jurnal*, **1-2:** 12 p.

Brickell C., Zuk J.D. (1996) A-Z Encyclopedia of Garden Plants. New York: DK Publishing Inc., USA, 1095 p.

Chryssavgi G., Vassiliki P., Athanasios M., Kibouris Th., Michael K. (2008) Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: Evaluation of antioxidant capacity of methanolic extracts. *Food Chemistry*, **107:** 1120-1130.

Ekundayo O., Laakso I., Holopainen M. et al. (1990) The chemical composition and antimicrobial activity of the leaf oil of *Vitex agnus-castus* L. *J. Essent. Oil Res*., **2(3):**115-119.

El-Kamari Fatima, Taroq A., El-Atki Y., Aouam I., Lyoussi B., Abdellaoui A. (2018) Chemical composition of essential oils from *Vitex agnus-castus* L. growing in Morocco and it's in vitro antibacterial activity against clinical bacteria responsible for nosocomial infections. *Asian J. Pharm Clin. Res.*, **11 (10**): 368–365.

Galletti G.C., Russo M.T., Bocchin P. (1996) Essential oil composition of leaves and berries of *Vitex agnus-castus* L. from Calabria, Southern Italy. *Rapid Commun. Mass Spectrum*, **10:** 1350-1345.

Hamid A.A., **Usman L.A.**, **Adebayo S.A.**, **Zubair M.F.**, **Elaigwu S.E.** (2010) Chemical constituents of leaf essential oil of north-central Nigerian grown *Vitex agnus-castus L. Advances in Environmental Biology*, **4(2):** 250-253.

Hüseyin F., Serhat E., Murat Ö. İ. Emrah D. (2014) Hayıt (*Vitex agnus-castus* L.)'da farklı toplama zamanlarının uçucu yağ oranı ve bileşenleri üzerine etkisi. *European Journal of Science and Technology*, **1(2):** 25-28,

Jennings W., Shibamoto T. (1980) Qualitative analysis of flavor and volatiles by glass capillary gas chromatography. New York: Academic Press rapid Manuscript Reproduction, 472 p.

Katiraee F., Mahmoudi R., Tahapour K., Hamidian G., Emami S.J. (2015) Biological properties of *Vitex agnus-castus L.* essential oil (Phytochemical component, antioxidant and antifungal activity). *Biotech Health Sci.*, **2(2):** 6 p.

Khalilzadeh E., Saiah G.V, Hasannejad H. et al. (2015) Antinociceptive effects, acute toxicity and chemical composition of *Vitex agnus-castus* L. essential oil. *Avicenna J. Phytomed*., **5:** 230-218.

Kotov M.I., Karnaukh E.D., Morozyuk S.S., Goncharov S.V. (1969) Essential oil plants of Ukraine. Kiev: Naukova Dumka, 102 p.

Oluwole O., Oladipupo A.L, Opeyemi A., Iresemowo O.O., Lawal O., **Iresemowo O.** (2012) Volatile constituents, antibacterial and insecticidal activities of essential oil from the leaves of *Vitex agnus-castus* L. (*Verbenaceae*). *Canadian Journal on Computing in Mathematics, Natural Sciences, Engineering and Medicine*, **3(7):** 256-260.

Senatore F., Reverchon E. (1996) Constituents of *Vitex-agnus-castus* L. essential oil. *Flavor Frag J.*, **11:** 179-182.

Tandon S., Mittal A.K., Pant A.K. (2008) Insect growth regulatory activity of *Vitex trifolia* and *Vitex agnus-castus* L. essential oils against *Spilosoma obliqua*. *Fitoterapia*, **79(4):** 283-286.

Tyagi T., Agarwal M. (2017) Phytochemical screening and GCMS analysis of bioactive constituents in the ethanol extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms. *Journal of Pharmacognosy and Phytochemistry*, **6 (1):** 195-206.

Ulukanli Z., Çenet M., Öztürk B., Bozok F., Karabörklü S., Demirci S.C. (2015) Chemical characterization, phytotoxic, antimicrobial and insecticidal activities of *Vitex agnus-castus L.* essential oil from East Mediterranean Region. *Journal of Essential Oil Bearing Plants*, **18(6):** 1500-1507.

Zahid H., Rizwani G.H., Ishaqe S. (2016) Phytopharmacological review on *Vitex agnus-castus L.* A potential medicinal plant. *Chinese Herbal Medicines (CHM)*, **8(1):** 67-71.

Zoghbi M.G.B., Andrade E.H.A., Maia J.G. (1999) The essential oil of *Vitex agnus-castus* L. growing in the Amazon region. *Flavor Fragrance Journal*, **14:** 211-213.

Abşeron şəraitində *Vitex agnus-castus* L. növünün yarpaqlarının efir yağının kimyəvi komponentləri

Məmmədova İradə Oruc qızı, Məmmədova Zümrüd Əmən qızı, Ələkbərli Ramiz Ələkbər oğlu

AMEA-nın Dendrologiya İnstitutu, Bakı, Azərbaycan

Dərman bitkiləri bütün dünyada istifadə olunur, çünki onların yan təsirləri riski daha azdır və kimyəvi dərmanlarla müqayisədə ekoloji cəhətdən təmiz, sərfəli istehsal, tədqiqatçıları daha çox araşdırma aparmağa həvəsləndirir. Geniş yayılmış dərman bitkilərindən biri, tropik və subtropik bölgələrdə yetişdirilən, müxtəlif sağlamlıq faydaları olan, *Vitex agnus-castus* L.-dir. Bu tədqiqatda Abşerona introduksiya edilmiş *Vitex agnus-castus* L. yarpaqlarının efir yağlarının kimyəvi tərkibi öyrənilmişdir. *Vitex agnus-castus* L.-nin təzə yarpaqlarından efir yağları hidrodistillə yolu ilə alınmış və onların uçucu komponentləri GC/MS ilə müəyyən edilmişdir. Dendrologiya İnstitutunda yetişdirilən Adi ərgudə yarpaqlarının efir yağının konsentrasiyası 0,45% olan, açıq sarı mayedir. Yarpaqların efir yağında 20-dən çox komponent müəyyən edilmişdir. Yarpaq yağında həmçinin çoxlu miqdarda 1,8-sineol (28,31%) və β-pinen (13,1%) vardir. Nəticələr, ölkəmizdə və dünyada eyni növlər üzərində aparılan tədqiqatların müqayisəsi əsasında, verilib.

Açar sözlər: *Vitex agnus-castus* L., *yarpaqlar, efir yağı, komponent tərkibi*

Химические компоненты эфирного масла листьев *Vitex agnus-castus* L., выращенных в условиях Апшерона

Мамедова Ирада Орудж кызы, Мамедова Зумруд Эмен кызы, Алекперли Рамиз Алекпер оглу

Институт дендрологии НАН Азербайджана, Баку, Азербайджан

Лекарственные растения используются во всем мире, потому что риск их побочных эффектов ниже, чем у химических лекарственных средств, а производство более экологично и рентабельно. Одним из наиболее распространенных лекарственных растений является *Vitex agnus-castus* L., который выращивается в тропических и субтропических регионах и обладает рядом преимуществ, способствующих сохранению здоровья человека. В данной работе изучен химический состав эфирных масел листьев *Vitex agnus-castus* L., интродуцированных на Апшерон. Эфирные масла из свежих листьев *Vitex agnus-castus* L. получали гидродистилляцией, а их летучие компоненты определяли методом ГХ/МС. Эфирное масло листьев витекс, выращенных в Институте дендрологии, представляет собой светло-желтую жидкость с концентрацией 0,45%. В этом эфирном масле идентифицировано более 20 компонентов. Оно также содержит большое количество 1,8-синеола (28,31%) и β-пинена (13,1%). Результаты основаны на сравнении исследований одних и тех же видов Vitex *agnus-castus* L, произрастающих в нашей стране и во всем мире.

Ключевые слова: *Vitex agnus-castus* L., *листья, эфирное масло, компонентный состав*

Journal of Life Sciences & Biomedicine, vol. 4(77), No 1, p. 53-67 (2022)

Mitochondrial dysfunction in kidney injury: potential therapeutic approaches

[1,2] Ahmadian Elham Asgar, [2,3,4] Khalilov Rovshan Ibrahimkhalil, [2,5] Eftekhari Aziz Mahammad

[1]Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Joint Ukraine-Azerbaijan International Research and Education Center of Nanobiotechnology and Functional Nanosystems, Drohobych, Ukraine & Azerbaijan
[3]Department of Biophysics and Biochemistry, Baku State University, 23 academician Zahid Khalailov, Baku 1148, Azerbaijan
[4]Institute of Radiation Problems, Azerbaijan National Academy of Sciences, 9 B.Vahabzade Str., Baku AZ1143, Azerbaijan
[5]Pharmacology and Toxicology Department, Tabriz University of Medical Sciences, Tabriz, Iran
For correspondence: *ahmadian.elham@yahoo.com; hrovshan@hotmail.com; eftekharia@tbzmed.ac.ir*

Received: April 07, 2022; Received in revised form: May 25, 2022; Accepted: May 27, 2022

Mitochondria are unique organelles that are essential for a variety of cellular functions, including ATP synthesis, calcium homeostasis, cell survival, and cell death. Mitochondria are an important source of energy production in eukaryotic cells and also play an important role in the production of lipids, nucleic acids, and amino acids. Mitochondria are vulnerable to oxidative stress. The main sources of ROS synthesis in cells are mitochondria and NADPH oxidase (NOX). In mitochondria, ROS is produced during the respiratory chain, while in NOX it is produced along the membranes of neutrophils and phagosomes. Under certain conditions, the production of free radicals such as $OH^{\bullet -}$**, and** $O_2^{\bullet -}$ **will result in vulnerabilities. ROS which is produced by mitochondria has many targets, including lipids, proteins, DNA, RNA, and mitochondrial DNA (mtDNA), which become a vulnerable target for oxidative stress due to the lack of histones. Impaired mtDNA and disrupted mitochondrial genome integrity have main roles in the development of severe early-onset and chronic aging-related diseases. It is becoming increasingly clear that long-term, tiny mtDNA damage is not only related to the aging process but may also be closely related to diabetes and nephropathies. Mitochondrial dysfunction has the main role in renal diseases. Epigenetic alterations and interactions between mtDNA, ROS, and inflammatory factors affect nephrons. Alterations of mtDNA affect the development and progression of chronic kidney disease. Alteration of mtDNA also has a significant role in nephropathies monitoring. Evidence suggests that modification in several mtDNA copies in the circulation and urine reflects mitochondrial dysfunction and kidney disease severity. This review will describe mitochondrial antioxidants in nephropathies therapy. Targeted mitochondrial antioxidants will become a new insight in nephropathies therapy. mtDNA can also be a therapeutic target.**

Keywords: *Mitochondrial, renal disease, mtDNA, oxidative stress*

INTRODUCTION

The kidney is a dynamic structure and has various physiological functions to maintain homeostasis. This dynamic structure is always affected by internal and external damage, which might impair the innate function of the kidney. But the same injury activates endogenous mechanisms to eliminate the injury, repair, and restore normal function. Lack of timely activation of renorestorative mechanisms or insufficient strength of these mechanisms leads to the accumulation of destructive factors and further tissue damage and the formation of a defective cycle in kidney function.

As the vicious cycle continues and more damage occurs over time, the normal function of the kidneys gradually decreases, and eventually disappears. To prevent and treat kidney damage, four

10.5281/zenodo.7239647
Available online 30 June 2022

points are important. The first is to know the macroscopic and microscopic structure of the kidney, the second is to understand the physiological and pathological mechanisms at the cell, organelle, and molecular levels, the third is to understand the restorative mechanisms and the fourth is the rapid therapeutic response. Time is an important factor in repairing, regeneration, and prevention of disease progression.

Mitochondrial structure

Mitochondria include an outer mitochondrial membrane (OMM) and an inner mitochondrial membrane (IMM), an intramembrane space (IMS), and a matrix (space between cristae). The matrix contains abundant proteins, mitochondrial DNA (mtDNA), and three types of RNA. Human mtDNA is a circular molecule with 16,569 pairs of bases. This molecule encodes 13 respiratory chain proteins, 2 rRNA molecules, and 22 tRNA molecules used to translate mitochondrial mRNA. All mtDNA transcripts and their translation products remain in the mitochondria, and all proteins encoded by mtDNA are made on mitochondrial ribosomes, and most proteins are located in the mitochondria (such as mtRNA polymerase) are made on cytoplasmic ribosomes (Kühlbrandt, 2015).

In addition to mtDNA replication, RNA and protein synthesis also occurs in mitochondria. This process is carried out by enzymes and molecules inside the organelle. The mtDNA locates in the mitochondrial matrix and is sometimes attached to IMM. The mitochondrial genome is about 510 times smaller than the nuclear genome. Mutations in mtDNA accumulate throughout the life of an organism, and the accumulation of mutations in mitochondrial genes over several decades might result in aging, degenerative disorders, and tumorogenesis (Falkenberg, 2018).

Respiratory chains are the major source of reactive oxygen species (ROS) in cells, so the contents of mitochondria (mainly the mitochondrial genome) are most exposed to ROS and its damage. In addition, the mtDNA replication system is less effective than the nuclear system in correcting errors resulting from replication and repair of mtDNA damage. As a result, these two factors create defects in mtDNA over time. According to one theory, this gradual accumulation of defects with age is the main cause of many signs of aging (such as decreased GFR). With an increasing percentage of defective mitochondria, energy production capacity decreases, and as cellular respiration rate drops, not only does energy production decrease but also the possibility of ROS production increases (Guo et al., 2018). Mitochondria fulfill a variety of functions in the cell, including the well-known energy production via oxidative phosphorylation. For this reason, the failure of mitochondria to operate correctly is associated with a wide spectrum of genetic disorders.

Energy production and maintenance of mitochondrial membrane potential

ATP is formed by the mechanism of oxidative phosphorylation in the respiratory chain of mitochondrial, which contains 5 protein complexes residing in IMM. The electrons produced during the oxidation of acetyl-CoA in the Krebs cycle are transferred to the intermediate molecules and then to the electron transfer chain in the IMM. As the electron passes through the electron transfer chain (ETC), the proton ions are pumped to the IMS and the electrochemical gradient of the proton ions from the IMS to the matrix is generated. Subsequently, ATP synthase phosphorylates ADP to form ATP by energy from the transfer of proton ions in the electrochemical gradient from IMS to the matrix (Cantó et al., 2015).

Respiratory chain function and ATP production depend on the integrity and stability of the IMM. The cardiolipin phospholipid in IMM has a crucial role in the formation of cristae, the proper curvature of the IMM, and the organization and placement of electron transfer chain complexes. Cytochrome C (Cyt C) as an electron carrier in the ETC is closely related to cardiolipin. When ROS increases, Cyt C acts as a peroxidase enzyme and oxidizes cardiolipin, which in turn leads to the disruption of SIMM integrity. Overproduction of ROS by impairing the integrity and stability of IMM reduces ATP production, opening the mitochondrial permeability transition pore (mPTP), the release of Cyt C (as an activator of mitochondrial apoptosis), and loss of mitochondrial membrane potential ($\Delta\Psi m$) and (Ascenzi et al., 2015).

MPV17 protein as an IMM resident protein has significant physiological effects on mitochon-

drial homeostasis. This protein, as a selective cation channel, controls the mitochondrial membrane potential (ΔΨm) as well as the passage of small molecules such as deoxynucleotides triphosphates (dNTPs). dNTPs are the building blocks of mtDNA. Genetic and structural abnormalities in the MPV17 protein prevent mtDNA repair and synthesis due to the reduction of dNTPs in mitochondria, which is directly related to mitochondrial biogenesis (Antonenkov et al., 2015; Dalla Rosa et al., 2016). MPV-like proteins are involved in ROS metabolism and modulation of apoptosis through functional and binding interaction with serine mitochondrial proteinases (Krick et al., 2008).

Mitochondrial Permeability Transition Pore (mPTPS) is a non-specific channel for the transmission of signals, materials, and ions. These channels regulate the potential of the mitochondrial membrane. CypD is an important regulator of mPTPS. Disruption of these channels leads to impaired ATP production and oxidative stress. mPTPS are physiologically open based on cellular requirements, but under pathological conditions they remain open for a long time, allowing the transfer of various substances (ions, proteins, and water). The mitochondrial membrane potential and ATP production capacity are lost through mPTPS due to unregulated discharge of substances (enzymes, electron transporters, ATP, and ADP), and also the mitochondria burst due to material entry and water osmosis (Kalani et al., 2018).

VDACsare the main carriers on OMM that cause bilateral salts to move between the cytoplasm and mitochondria. VDACs improve renal morphology and renal function by increasing mitochondrial respiratory capacity, reducing mitochondrial fission, improving mitochondrial function and dynamics, and increasing cell survival after acute renal injury (AKI) (Nowak et al., 2020). Mitochondrial outer membrane permeability, release of pro-apoptotic BCl-2 family proteins, loss of mitochondrial inner membrane potential, mitochondrial swelling, and disruption of mitochondrial structure leading to eventual lysis of mitochondria.

Role of mitochondria in oxidative stress

The oxygen molecule is the final electron receptor in the mitochondrial electron transfer chain. In the ETC, 2 water molecules are made by giving 4 electrons to an oxygen molecule and adding 4 protons to it. Mitochondria are the source of energy (ATP) production in the cell. In the process of oxidative phosphorylation, the oxygen molecule is reduced by receiving 4 electrons and producing water. Meanwhile, about 0.4 to 4% of the oxygen molecule is not reduced. So the oxygen molecule is incompletely reduced by taking an electron and becomes an oxygen radical called superoxide anion (O_2●-). Although superoxide anion has a short half-life and is an unstable molecule, it is a strong oxidizing agent and can oxidize all molecules (lipids, proteins, and nucleic acids) in the cell. The superoxide anion (enzymatically and non-enzymatically) is converted to a peroxide anion ($O_2^{\bullet 2-}$) by taking another electron, which is slightly more stable. The peroxide anion is converted to hydrogen peroxide (H2O2) by taking 2 protons. Under physiological conditions, superoxide anions are converted to peroxide anion or hydrogen peroxide (H_2O_2) by the enzyme superoxide dismutase (SOD). Subsequently, H_2O_2 is converted to water by the enzyme catalase and glutathione peroxidases (GPX) (Kirkham and Rahman, 2006).

Under pathological conditions, hydrogen peroxide produces one electron, one hydroxyl radical (OH●-), and one hydroxyl anion (OH-). The hydroxyl radical is a strong oxidant and can oxidize any organic matter in the cell, and the hydroxyl anion in the liquid is converted to water by taking a proton. The superoxide anion reacts with nitric oxide (NO) to generate the peroxynitrite radical (ONOO-), and the hydroxyl radical can react with the chlorine anion (Cl-) to form hypochlorous acid (HOCl), which is also a strong oxidizer. Molecules formed by the placement of an unpaired electron in an oxygen molecule are called oxygen-free radicals. Superoxide anion, peroxide anion, hydrogen peroxide, peroxynitrite, and hypochlorous acid are oxygen free radicals and are highly reactive molecules, hence they are called reactive oxygen species (ROS) (McBride et al., 2006).

Physiologically, in various cellular processes, free radicals or oxidants are produced, each of which has a specific physiological function, and on the other hand, their excess amounts are neutralized by the endogenous antioxidant system. So in the body, there is a real balance between the oxidant system and the antioxidant. Under pathologi-

cal conditions, the production of oxidants increases, and there is not enough antioxidant power to neutralize them. Because of this, oxidants cause a lot of damage to various systems in the body and cause various diseases such as kidney disease (Turrens, 2003).

The most important part of the oxidative system in the body is ROS. An imbalance between the oxidant and antioxidant systems is called oxidative stress, and it is the pressure that oxidants exert on the body. Antioxidant systems or agents contain compounds that can protect the body against the harmful effects of oxidative stress (active oxygen and nitrogen species). Antioxidants play an important role in inhibiting the active species of oxygen and nitrogen and preventing their formation. Endogenous antioxidants are divided into three categories: 1) enzymatic systems such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, 2) small molecules such as vitamin C, uric acid, glutathione, and vitamin E, and 3) Proteins such as albumin, transferrin, ceruloplasmin, and metallothioneins (Valko et al., 2007). Endogenous non-enzymatic antioxidants include: thioredoxin (Trx), glutathione (GSH), α-Lipoic acid (1,2-dithiolane-3-pentanoic acid), melatonin (N-acetyl-5-methoxytryptamine), and coenzyme Q10 and exogenous non-enzymatic antioxidants include: vitamin C, curcumin, resveratrol, quercetin, vitamin E, lycopene, polyphenols, carotenoids, epigallocatechin-3-gallate (EGCG), flavonoids, organic sulfur compounds and several minerals (selenium, copper, zinc, and manganese) (Mailloux, 2018; Sharifi-Rad et al., 2020). Oxidants are always produced in the body and must always be eliminated, so continuous use of antioxidants is recommended.

In pathological conditions, with increasing ROS and decreasing antioxidant factors, ROS accumulates in the mitochondria and directly damages its structure and components. Also, due to the increased permeability of the mitochondrial membrane caused by ROS, ROS leaks into the cytoplasm and in addition to direct damage to cellular structures, activates inflammation, apoptosis, and harmful signaling pathways. Inhibition of mitochondrial oxidants with antioxidant and renoprotective agents is a specific goal in preventing mitochondrial damage and subsequent prevention of injury and progression of kidney disease (Irazabal and Torres, 2020).

Various experimental models showed that with increasing oxidant factors, endogenous antioxidant factors decrease. Therefore, by using exogenous antioxidants, the destructive effects of oxidative stress can be neutralized. These studies showed that the mitochondrial antioxidant defense system is disrupted before the onset of kidney damage. Therefore, by increasing the antioxidant power and maintaining the proper mitochondrial function, further tissue damage can be prevented and the healing process of kidney disease can be accelerated.

Mitochondrial antioxidants include Szeto-Schiller peptide (SSP), MitoQ, and plastoquinone analogs (SkQ1/SkQR1), which accumulate in the mitochondrial matrix and interact with cardiolipin. Cardiolipin is the main constituent of IMM and plays a major role in maintaining the potential of the mitochondrial membrane (Birk, Chao, et al. 2015). SSPs enhance ATP synthesis, decrease electron leakage and ROS formation, prevent cardiolipin peroxidation, and prevent the consequences of mitochondrial impairment, including inflammation apoptosis, (Szeto 2017). In particular, SSP, MitoQ, MitoTEMPO, and SkQR1 protect against kidney damage in experimental models by reducing oxidative damage and inflammation (Xiao et al., 2017).

Mitochondrial biogenesis

The number and content of mitochondria are determined by the metabolic needs of the cell. The defective mitochondria are selectively removed and new mitochondria are created by fusion and fission mechanisms. The molecular mechanism of mitochondrial biogenesis needs a close relationship between mitochondria and the nucleus. The regulatory factors of biogenesis are peroxisome proliferator-activated receptor (PPAR), PPARγ coactivator 1α (PGC-1α), sirtuin-1 and 3 (SIRT1/3) family deacetylase, AMP-activated protein kinase (AMPK), and nuclear respiratory factors 1 and 2 (NRF1 and NRF2) (Quirós et al., 2016). The action of PGC-1α (as an abiogenesis stimulant) is regulated by AMPK-induced phosphorylation and SIRT1-induced deacetylation (Tran et al., 2016). PGC-1α activates the expression of genes and pro-

teins involved in mtDNA replication and transcription, electron transfer chain, and oxidative phosphorylation mechanism by modulating the expression of nuclear transcription factors NRF1 and NRF2 (Zoja et al., 2014).

Lack of oxygen and metabolic disorders activate the transcription factor FOXO3 by inducing stress in renal tubular epithelial cells. Activated FOXO3 reduces cell damage and increases cell survival by stimulating autophagy and neutralizing ROS in renal tubular cells. FOXO3 induces autophagy by enhancing the expression of Atg proteins and neutralizing ROS by increasing the expression of antioxidant factors. Prolyl hydroxylase (PrH) by hydroxylation degrades FOXO3 through the proteasomal pathway of ubiquitin. Hypoxia and metabolic disorders inhibit the action of PrH hydroxylation on FOXO3 (Lin, 2020; Quan et al., 2020).

Under physiological conditions, mitochondrial biogenesis takes two forms: fission and fusion. In infusion mode, two mitochondria join together to form larger mitochondria with larger content. But in the case of fission, one mitochondrion turns into two smaller mitochondria with a smaller volume and lower content. Over time and according to metabolic needs, the internal content of mitochondria increases.

In pathophysiological and abnormal conditions, mitochondrial biogenesis occurs mainly in the form of fission, and over time and with the disorder, the mitochondrial content does not increase. Unfortunately, the new mitochondria cannot produce enough energy and will also act as a hub for ROS production.

Two points are important in the production of new mitochondria. First, the production of new mitochondria should be accompanied by a surge in enzyme content to increase metabolic activity, and second, the production of new mitochondria should be accompanied by an increase in protective content to counteract the activity of oxidants. Therefore, mitochondria without sufficient content (metabolic enzymes and antioxidants) will not be able to produce energy, and also the lack of antioxidants along with the production of oxidants will cause more oxidative damage.

Mitochondrial dynamics

The constant change in the number, size, location, and protein content of mitochondria based on the energy needs of the cell is called mitochondrial dynamics. This dynamic is essential for size, morphology, function, energy production, maintenance of homeostasis, and cell survival (Liesa and Shirihai, 2013). Mitochondrial dynamics control proteins include fission proteins (the large GTPase, dynamin-related protein 1 (DRP1), and mitochondrial fission protein (Fis1)), and fusion proteins (mitofusins 1 and 2 (Mfn1, Mfn2) and optical atrophy 1 (OPA1)) (Zhan et al., 2013).

Sirtuin deacetylases (SIRT1/3) regulate mitochondrial dynamics and function. Matrix-dwelling sirtuins activate the fusion process by deacetylation of fusion proteins and improve mitochondrial function in acute kidney disease (AKI) (Morigi, Perico, et al. 2015). Disruption of mitochondrial dynamics causes tissue damage and renal disease (Benigni et al., 2016).

In the physiological state, Dynamin-related protein 1 (DRP1) controls mitochondrial function by having GTPase as a fission agent. But DRP1 is activated in response to a decrease in ATP or damage to the epithelial cells of the renal tubule and accumulates in the mitochondria, leading to mitochondrial fission and apoptosis. In experimental models, the removal of Drp1 and the use of DRP1 inhibitor (mdivi-1) protect the kidney against kidney damage and improve its function (Perry et al., 2018). And also the interaction between mitofusin 2 (Mfn-2) in the endoplasmic reticulum and Mfn-1 or Mfn-2 in OMM regulates the relationship between the two organelles (Xia et al., 2019).

Mitochondrial mitophagy

The destruction of organs and defective cellular material by lysosomes is called autophagy. In this process, by forming an autophagosome, the contents of the cytoplasm, damaged organs, and dysfunctional proteins are broken down and used as nutrients in the cell. Physiological autophagy in starvation conditions is necessary for ATP production, amino acid recycling, and protein synthesis, and also in these situations, autophagy is a key mechanism in the removal of toxic cellular substances, defective organelles, and the formation of

damage-free cells. Mitophagy is a type of autophagy in which disturbed mitochondria are removed from the cell. Injured mitochondria are encapsulated in autophagosomes and decompose in autolysosomes by losing the potential of the inner membrane (Higgins and Coughlan, 2014).

Mitophagy is a complex mechanism controlled by several signaling pathways, kinase proteins, and mitochondrial proteins that regulate dynamics and transmission. Pathological mitophagy is dependent on ROS and is activated under oxidative stress. Disorders of mitophagy mechanisms lead to kidney disease (Duann et al., 2016).

Mitochondria and cell death

The two main mechanisms of cell death are apoptosis and necrosis, which differ in the type of operation. Apoptosis is a physiological and/or pathological cell death that occurs during certain stimuli. Necrosis, on the other hand, is merely pathological cell death and occurs during severe cell injury such as hypoxia and external toxins. Necrosis is a passive process and occurs in the absence of ATP, while apoptosis is an active process and is dependent on ATP energy.

The process of apoptosis occurs through two internal and external cellular pathways. The external pathway begins with the binding of important ligands such as TNFα and Fas to death-inducing membrane receptors. While the internal pathway (mitochondrial pathway), the main pathway of apoptosis, is associated with alteration in mPTP and liberation of apoptotic agents.

Elevated permeability of the OMM in response to cell death signals releases proapoptotic molecules from the IMS into the cytoplasm, such as apoptosis-inducing factor (AIF) and Smac/DIABLO. The latter has an antagonistic effect on the inhibitors of caspase. AIF normally plays an antioxidant role in mitochondria. AIF released from mitochondria during the process of apoptosis causes damage to nuclear DNA in a caspase-independent pathway. Release of cytochrome c is a common occurrence in apoptosis due to the opening of the mitochondrial permeability transition pore, the presence of a channel-specific for cytochrome c in the outer mitochondrial membrane, swelling, and rupture of the outer mitochondrial membrane (Galluzzi et al., 2018).

However, from the molecular point of view, apoptosis is mainly determined by the balance between the specific regulatory proteins of pre-apoptosis and anti-apoptosis. These include Bax and Bcl- 2 proteins, which are involved as major proteins in the formation of mitochondrial apoptosis. Bax protein releases apoptotic agents such as cytochrome c from the IMS into the cytosol by decreasing the stability and increasing the permeability of the outer mitochondrial membrane. In contrast, Bcl-2 protein preserves the integrity of the mitochondrial membrane by counteracting the apoptotic activity of Bax proteins. In the endogenous or mitochondrial pathway, caspase-9 is an initiator of caspase and also the common denominator of all apoptotic pathways is ultimately the stimulation of caspase-3 and the breakdown of vital cell proteins (Havasi and Borkan, 2011).

Under physiological conditions, Cytochrome C (Cyt C) is freely present in the matrix or binds to them by attaching to cardiolipin and acting as an electron carrier between respiratory complexes III and IV of the ETC. ETC With mitochondrial damage, production, and accumulation of ROS lead to the conversion of cytochrome c to peroxidase, which oxidizes cardiolipin and releases Cyt C from the matrix to the cytosol. Which eventually activates caspase and apoptosis (Wan et al., 2019).

Mitochondria and cell proliferation and differentiation

Cellular proliferation and differentiation upon damages are considered important repair mechanisms. Dynamic changes in morphology as well as in mitochondrial content play an essential role in mediating cell cycle events. During the transition from phase G1 to phase S of the cell cycle, mitochondria form an extensive network of interactions with different cell components. By reducing the potential of the mitochondrial membrane, the progression of phase G1 to phase S can be prevented. By using an uncoupling agent (carbonyl cyanide-4-phenylhydrazone) in rat renal epithelial cells, cell cycle progression can be prevented by reducing the mitochondrial membrane potential. DRP1 is the major regulator of mitochondrial fission and by inhibiting it; the cell cycle in the S phase is stopped (Mitra et al. 2009).

Mitophagy and mitochondrial clearance appear to be crucial for cell differentiation and cell proliferation along with changes in energy production from oxidative phosphorylation to anaerobic metabolism. In the repair of renal tubules after ischemia-reperfusion injury (IRI), the glycolytic capacity of the cell increases by decreasing the number of mitochondria (Pennock et al., 2015).

Energy production and mtROS formation mainly control mitochondrial-related cell proliferation and differentiation. During the transition from phase G1 to phase S due to DNA replication and energy requirements, energy production by mitochondria increases but in the transition from phase G2 to phase M due to a compensatory increase in Glycolysis or reduction of energy demand, the number of mitochondria of the cell is reduced by degradation (Thomasova and Anders, 2014).

In the repair process, the amount of ROS in the cell is inversely related to cell proliferation and differentiation. Thus, ROS enhances cell proliferation and differentiation at physiological concentrations and inhibits at pathological concentrations. ROS in physiological concentrations modulates cell proliferation and differentiation by regulating many transcription factors such as nuclear factor-kB, hypoxia-1 inducing factor, and different protein kinases. Also, ROS in pathological concentrations causes apoptosis and cell death by activating apoptotic pathways and stopping the cell cycle by p53 (Hamanaka and Chandel, 2010; Antico Arciuch et al., 2012).

The cyclin B1/Cdk1 protein complex plays a fundamental role in regulating cell division and mitochondrial activity. This complex is an excellent target for controlling cellular energy production that can promote progress in restorative therapies. The cyclin B1/Cdk1 protein controls the G2 phase of the cell cycle. As the cell cycle continues to mitosis, the cyclin B1/Cdk1 complex migrates from the nucleus to the mitochondria, increasing energy production by phosphorylating mitochondrial proteins (Wang, Fan, et al. 2014). Cdk1 is also activated by pro-apoptotic proteins (Bax and Bak). Activated Cdk1 migrates to mitochondria and induces apoptotic cell death in mitochondria by phosphorylation of anti-apoptotic proteins (Bcl-2 and Bcl-xL) (Darweesh et al., 2021).

Mitochondria and inflammatory response

Inflammation has evolved as a set of complex mechanisms overtime to maintain homeostasis and is a defensive process that protects the body against internal and external pathogens. Excessive strengthening of inflammatory mechanisms in physiological and pathological conditions is harmful and damages the body.

Table 1. Therapeutic targeting of the cyclic GMP–AMP synthase (cGAS) and stimulator of interferon genes (STING) pathway

cGAS inhibitor compounds		STING inhibitor compound	
Catalytic site inhibitors	PF-06928215, RU.365, RU.521, G 150, Compound S3,	STING antagonists targeting the CDN-binding site	Tetrahydroisoquinolines (Screening hit Compound 1, Compound 18)
			Astin C
Inhibitors that disrupt DNA binding	Antimalarial drugs (Hydroxychloroquine, Quinacrine, X6)	Targeting STING palmitoylation sites	Nitrofurans (C-176 and C-178, C-170 and C-171) Indole ureas (H-151)
	Suramin		Nitro fatty acids (NO2-cLA and NO2-A)
	Suppressive oligodeoxynucleotides (A151)		Acrylamides (BPK-21and BPK-25)
Compounds with the undetermined mechanism of action	CU-76	Inhibitors of unknown mechanism	Compound 13

Under normal circumstances, the inflammatory process is critical for tissue repair after injury. Nevertheless, persistent and prolonged inflammation exists in a range of chronic disorders including diabetes and CKD. Mitochondria is tightly connected with the regulation of inflammation during kidney injury and act as a proinflammatory signaling center. mtROS is an activator of inflammation and increases the expression of proinflammatory genes. Various molecular forms associated with mitochondrial damage, such as formyl peptides and mtDNA, can bind to Toll-like receptors or NOD-like receptors and lead to inflammation (West et al., 2011).

Mitochondrial damage in renal tubular cells by mtDNA leakage through BAX-activated pores in OMM to the cytosol by activating the cGAS-STING pathway (As a sensor sensitive to cytosolic DNA) causes inflammation of the kidney and AKI progress. Inherently, this pathway can detect viral and bacterial DNA and induce immune and inflammatory responses in infected cells, so the prohibition of the cGAS-STING pathway could be an effective therapeutic target in mitochondrial injury and inflammatory kidney disease (Table 1) (Maekawa et al., 2019; Decout et al., 2021). However, activation of this pathway is especially important in resistance and elimination of external pathogens by activating inflammation.

Mitochondria and kidney damage and/or repair

The kidney plays a crucial role in maintaining the homeostasis of the internal environment. Physiological function of the kidney involves three mechanisms: glomerular filtration, reabsorption, and tubular secretion. Through these three mechanisms, the kidneys excrete metabolic wastes and retain nutrients, water, and electrolytes, and regulate osmolality, pH, and arterial blood pressure (Suliman and Piantadosi, 2016).

Active reabsorption of nutrients for the body leads to high energy demand for the kidneys, which is provided only by mitochondrial oxidative metabolism. Active reabsorption occurs mostly in the proximal tubules. These tubules have limited capacity for glycolysis and the main mechanism of ATP production in them is oxidative-phospho-rylation (aerobic respiration) (Lan et al., 2016).

Proximal tubules have many mitochondria, and maintenance of mitochondrial function and homeostasis is essential for physiological renal function. AKI is characterized by a sudden decline in kidney function, often caused by stress, ischemia-reperfusion (IR), sepsis, or nephrotoxins.

AKI is related to high mortality and significantly promotes the development of CKD (5). The results of empirical and clinical studies showed that improper repair after AKI leads to interstitial tubular fibrosis and eventually to CKD. On the other hand, one of the causes of the pathogenesis of kidney damage in AKI and CKD is mitochondrial damage or dysfunction (See et al., 2019).

After the injury, the surviving tubular cells proliferate and migrate to the affected area. The cells in the affected area differentiate and become fully mature tubular cells, and then the process of repairing the damaged renal tubules begins. Mild damage in kidney disease heals quickly by activating nonrestorative processes. While severe and progressive damage in kidney disease with a defective repair process leads to the destruction of nephrons and the development of kidney diseases such as interstitial tubular fibrosis and advanced CKD (Nath et al., 1998).

Transient receptor potential ankyrin 1 (TRPA1) is expressed in renal tubular epithelial cells. In experimental models, TRPA1 increases mitochondrial homeostasis and reduces renal damage by inhibiting fission, inducing fusion, and reducing oxidative stress, and inflammation (Zhu et al., 2018). TRPA1 is elevated in the renal tubules of patients with acute tubular necrosis (ATN) and its tubular expression is directly related to the severity of tubular and renal damage. Tubular TRPA1 is therefore a risk factor for improving renal function in acute tubular necrosis (Dembla et al., 2016; Wu et al., 2019). These effects probably suggest that TRPA1 does not play an independent role in the development of kidney disease but is an intermediary of major mechanisms involved in renal injuries such as increased oxidative stress and inflammation.

The mechanisms involved in kidney damage and repair are complex and consist of several different factors including vascular, tubular, and inflammatory factors as well as different signaling pathways. Understanding how these factors interact is a turning point in repairing and rehabilitating a damaged kidney.

Table 2. Targeting mitochondrial mechanisms for the treatment of kidney disease

Therapeutics	Chemicals	Action mechanisms
Antioxidants		
MitoQ, MitoTEMPO, MitoE, Mito-CP, plastoquinone analogs (SkQ1/SkQR1), Szeto–Schiller (SS) peptides	Mitoquinone	Accumulation of anti-oxidants at matrix in a ΔΨm-dependent manner; ROS scavenger
Biogenesis inducers		
Resveratrol (SRT501)	Small peptides	AMPK/SIRT-1/PGC-1α axis activator
AICAR		SIRT3 activator, an AMPK agonist
Formoterol		β2-adrenoceptor agonist, enhancing PGC-1α synthesis
ROS metabolism and bioenergetics		
Mitochonic acid (MA-5)	Synthetic compound	OXPHOS-independent increase of ATP synthesis, reduce ROS
Cardiolipin protection		
Bendavia (SS-31)	Szeto-Schiller tetrapeptide	Protect cardiolipin from peroxidation; increase ATP and reduce ROS
		Prevent cyt C transformation into the peroxidase
mPTP inhibitor		
cyclosporin A (CsA)	Small molecule	Interact with cyclophilin D (an mPTP structural protein)
TDZD-8		mPTP inhibitors, GSK3β inhibitor
Fission inhibitor		
Mdivil	Small molecule	Inhibitor of mitochondrial division; induce mitochondria fusion
		Block DRP1 assembly and translocation
K_{ATP} channel opener		
Simdax (Levosimendan)	Small molecule	Calcium sensitizer, K_{ATP} channel opener
cyt C assembly		
KLF6	DNA-binding zinc finger transcriptional regulator	enhance cyt C assembly

Abbreviation: Mitochondrial inner membrane potential (ΔΨm), oxidative phosphorylation (OXPHOS), AMP-activated protein kinase (AMPK), mitochondrial permeability transition pore (mPTP), Glycogen synthase kinase 3β (GSK3β), Dynamin related protein 1 (DRP1)

Kidney damage may alter mitochondrial structure and function in a variety of ways, including changes in calcium homeostasis, membrane integrity, ROS formation, mitochondrial transport, biogenesis, dynamics (fusion/fission), and mitophagy. disruption of mitochondrial homeostasis might in turn cause further kidney damage and cause a detrimental feedback loop (Brooks, Wei, et al. 2009).

Henle ascending thick tubular cells and proximal medullary cells have high mitochondrial due to high energy demand for reabsorption of salts and substances necessary for the body (Weidemann and Krebs, 1969). The main source of energy in tubular cells is ATP production from fatty acid β-oxidation (FAO) in the mitochondria, which is mediated by carnitine palmitoyl-transferase 1 (CPT1) as a restriction enzyme. Therefore, tubular cells are very sensitive to kidney damage due to a lack of energy (ischemic injury and oxidative stress) (Wang and Youle 2009).

Mitochondria is an important factor in various cellular processes such as regulating catabolism and anabolism, maintaining calcium homeostasis and redox potential, and regulating signaling pathways for cell survival and death. Therefore, maintaining the integrity and physiological functions of mitochondria is vital for cellular homeostasis. Mitochondria and their mechanisms have evolved to make the cell and its organs resistant to injury and stress. These mechanisms operate at three different cellular, organelle, and molecular levels and include antioxidant resistance, control of metabolites and messenger molecules, transcription and repair of mtDNA, mitochondrial dynamics (fusion and fission), and mitochondrial biogenesis, and mitophagy (Bhargava and Schnellmann, 2017). Injury and dysfunction of mitochondria lead to cell death, tissue damage, and failure.

Mitochondria play a key role in the mechanisms involved in kidney damage and repair. Mi-

tochondrial pathological changes are evident before and after renal dysfunction. Protecting and improving mitochondrial function before the onset of kidney injury and dysfunction is an important goal in preventing further injury and treating primary injuries. Protecting and restoring mitochondrial function under stress and disease by using genetic or pharmacological renoprotective agents prevents damage and disease progression (Perry et al., 2018).

Mitochondrial damage is typically caused by AKI by sepsis, IRI, and renal toxicity in experimental models mitochondrial damage in AKI is in connection with mitochondrial fragmentation, decreased mitochondrial mass, mitochondrial swelling, and cristae dysfunction, apoptosis, and generally mitochondrial dysfunction (Heidari et al., 2018).

Mitochondria and subsequent renal injury can be treated by increasing mitochondrial function by improving ETC function, biogenesis, and increasing FAO, or by reducing the cellular effects of mitochondrial dysfunction by reducing ROS formation, inflammation, apoptosis, pyroptosis, and autophagy (Tábara et al., 2014).

Renal cancer

Cell culture and in vivo experiments demonstrated that mitochondrial dysfunction is responsible for increased survival of renal cancer cells and cancer metastasis. Bisulfite sequencing analysis revealed that the downregulation may occur by DNA hypermethylation of CpG islands in the promoter regions in renal cancer cells. Taken together, these findings suggested that renal cancer cells acquire additional anti-apoptotic ability through the DNA methylation during the interactions with renal microenvironment, which may in turn lead to the cancer progression and metastasis.

CONCLUSION

Kidney diseases are one of the health problems of the world community and many people are affected by them. The kidney is an active and dynamic organ and is very prone to damage. AKI is caused by infection, toxic substances, and dehydration. If these acute injuries are not repaired and treated, it can lead to deteriorating kidney function and health. As kidney disease progresses, the acute condition may become chronic and lead to CKD. Chronic diseases reduce kidney function over time by creating a vicious-positive cycle. Severe acute injuries and prolonged chronic injuries cause complete loss of kidney function and the final stage of kidney disease, which has no treatment other than dialysis and new kidney transplantation.

For the treatment of kidney injuries, different parts and mechanisms of the kidney at the cellular, organelle, and molecular level can be selected as targets. One of the selected targets is mitochondria, which is the most important center of energy production in the cell. Because cells need enough energy to maintain homeostasis, survive, and prevent injury, targeting the components and mitochondrial signaling pathways can be an appropriate treatment for kidney disease. Interestingly, before the symptoms of kidney disease appear, mitochondrial lesions and disorders appear on an electron microscopic level.

Although it is important to discover the processes and mechanisms that cause kidney disease, it seems that the best treatment in experimental studies is the use of combination therapies. Because tissue damage is widespread in the disease and affects different parts of the cell. Focusing on one cell part and one mechanism may not be very helpful in discovering new therapies.

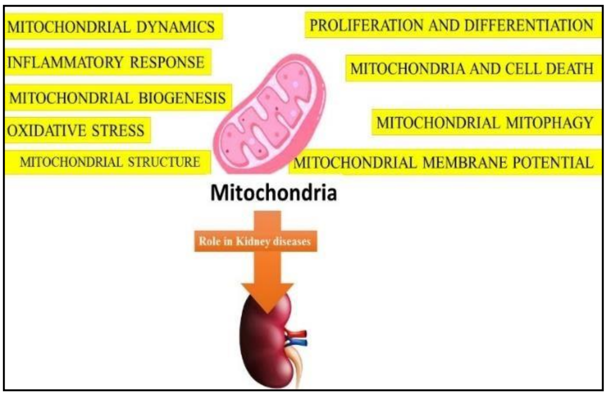


Fig. various factors can be used to inhibit destructive processes.

For example, in the treatment of acute kidney disease, in addition to reducing voluntary activities to reduce kidney function and reduce the need for energy production in the mitochondria, various

factors can be used to inhibit destructive processes (such as oxidative stress, signaling pathways of inflammation and cell death) and stimulate repair processes (e.g. Biogenesis, survival, and repair signaling pathways) (see: Figure). So far, many renoprotective and renorestorative agents have been discovered, but it will take a long time to introduce an effective treatment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Antico Arciuch V.G., Elguero M. E., Poderoso J. J., Carreras M.C. (2012) Mitochondrial regulation of cell cycle and proliferation. *Antioxid. Redox Signal.*, **16(10):** 1150-1180.

Antonenkov V.D., Isomursu A., Mennerich D. et al. (2015) The Human mitochondrial DNA depletion syndrome gene MPV17 encodes a non-selective channel that modulates membrane potential. *J. Biol. Chem.*, **290(22):** 13840-13861.

Ascenzi P., Coletta M., Wilson M. T., Fiorucci L. et al. (2015) Cardiolipin-cytochrome c complex: Switching cytochrome c from an electron-transfer shuttle to a myoglobin- and a peroxidase-like heme-protein. *IUBMB Life*, **67(2):** 98-109.

Benigni A., Perico L., Macconi D. (2016) Mitochondrial dynamics is linked to longevity and protects from end-organ injury: The emerging role of sirtuin 3. *Antioxid. Redox Signal.*, **25(4):** 185-199.

Bhargava P., Schnellmann R.G. (2017) Mitochondrial energetics in the kidney. *Nat. Rev. Nephrol.*, **13(10):** 629-646.

Birk A.V., Chao W.M., Liu S., Soong Y., Szeto H.H. (2015) Disruption of cytochrome c heme coordination is responsible for mitochondrial injury during ischemia. *Biochim. Biophys. Acta*, **1847(10):** 1075-1084.

Brooks C., Wei Q., Cho S.G., Dong Z. (2009) Regulation of mitochondrial dynamics in acute kidney injury in cell culture and rodent models. *J. Clin. Invest.*, **119(5):** 1275-1285.

Cantó C., Menzies K.J., Auwerx J. (2015) NAD(+) metabolism and the control of energy homeostasis: A balancing act between mitochondria and the nucleus. *Cell Metab.*, **22(1):** 31-53.

Dalla Rosa I., Cámara Y., Durigon R. et al. (2016) MPV17 loss causes deoxynucleotide insufficiency and slow DNA replication in mitochondria. *PLoS Genet.*, **12(1):** e1005779.

Darweesh O., Al-Shehri E., Falquez H., Lauterwasser J., Edlich F.and Patel R. (2021) Identification of a novel Bax-Cdk1 signalling complex that links activation of the mitotic checkpoint to apoptosis. *J. Cell. Sci.*, **134(8)**.

Decout A., Katz J.D., Venkatraman S., Ablasser A. (2021) The cGAS–STING pathway as a therapeutic target in inflammatory diseases. *Nature Reviews Immunology*, **21(9)**:548-69.

Dembla S., Hasan N., Becker A., Beck A., Philipp S.E. (2016) Transient receptor potential A1 channels regulate epithelial cell barriers formed by MDCK cells. *FEBS Lett.*, **590(10):** 1509-1520.

Duann P., Lianos E. A., Ma J., Lin P.H. (2016) Autophagy, innate immunity and tissue repair in acute kidney injury. *Int. J. Mol. Sci.*, **17(5).**

Falkenberg M. (2018) Mitochondrial DNA replication in mammalian cells: overview of the pathway. *Essays Biochem.*, **62(3):** 287-296.

Galluzzi L., Vitale I., Aaronson S.A. et al. (169 authors) (2018) Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ.*, **25(3):** 486-541.

Guo R., Gu J., Zong S., Wu M., Yang M. (2018) Structure and mechanism of mitochondrial electron transport chain. *Biomed. J.*, **41(1):** 9-20.

Hamanaka R.B., Chandel N.S. (2010) Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. *Trends Biochem. Sci.*, **35(9):** 505-513.

Havasi A., Borkan S.C. (2011) Apoptosis and acute kidney injury. *Kidney Int.*, **80(1):** 29-40.

Heidari R., Ahmadi A., Mohammadi H., Ommati M.M., Azarpira N., Niknahad H. (2018) Mitochondrial dysfunction and oxidative stress are involved in the mechanism of methotrexate-induced renal injury and

electrolytes imbalance. *Biomed. Pharmacother*., **107:** 834-840.
Higgins G.C., Coughlan M.T. (2014) Mitochondrial dysfunction and mitophagy: the beginning and end to diabetic nephropathy? *Br. J. Pharmacol.*, **171(8):** 1917-1942.
Irazabal M.V., Torres V.E. (2020) Reactive oxygen species and redox signaling in chronic kidney disease. *Cells*, **9(6)**.
Kalani K., Yan S.F., Yan S.S. (2018) Mitochondrial permeability transition pore: a potential drug target for neurodegeneration. *Drug Discov. Today*, **23(12):** 1983-1989.
Kirkham P., Rahman I. (2006) Oxidative stress in asthma and COPD: antioxidants as a therapeutic strategy. *Pharmacol. Ther.*, **111(2):** 476-494.
Krick S., Shi S., Ju W. et al. (2008) Mpv17l protects against mitochondrial oxidative stress and apoptosis by activation of Omi/HtrA2 protease. *Proc. Natl. Acad. Sci.* USA **105(37):** 14106-14111.
Kühlbrandt W. (2015). Structure and function of mitochondrial membrane protein complexes. *BMC Biology*, **13(1):** 89.
Lan R., Geng H., Singha P.K. et al. (2016) Mitochondrial pathology and glycolytic shift during proximal tubule atrophy after ischemic AKI. *J. Am. Soc. Nephrol.*, **27(11):** 3356-3367.
Liesa M., Shirihai O.S. (2013). "Mitochondrial dynamics in the regulation of nutrient utilization and energy expenditure. *Cell Metab*., **17(4):** 491-506.
Lin F. (2020) Molecular regulation and function of FoxO3 in chronic kidney disease. *Curr. Opin. Nephrol. Hypertens.*, **29(4):** 439-445.
Maekawa H., Inoue T., Ouchi H. et al. (2019) Mitochondrial damage causes inflammation via cGAS-STING signaling in acute kidney injury. *Cell Rep*., **29(5):** 1261-1273.e1266.
Mailloux R.J. (2018) Mitochondrial antioxidants and the maintenance of cellular hydrogen peroxide levels. *Oxidative Medicine and Cellular Longevity*, **2018:** 7857251.
McBride H.M., Neuspiel M., Wasiak S. (2006) Mitochondria: more than just a powerhouse. *Curr. Biol*., **16(14):** R551-560.
Mitra K., Wunder C., Roysam B., Lin G., Lippincott-Schwartz J. (2009) A hyperfused mitochondrial state achieved at G1-S regulates cyclin E buildup and entry into S phase. *Proc. Natl. Acad. Sci. U S A*, **106(29):** 11960-11965.
Morigi M., Perico L., Rota C. et al. (2015) Sirtuin 3-dependent mitochondrial dynamic improvements protect against acute kidney injury. *J. Clin. Invest.*, **125**(2): 715-726.
Nath K.A., Grande J.P., Croatt A.J., Likely S., Hebbel R.P., Enright H. (1998) Intracellular targets in heme protein-induced renal injury. *Kidney Int*., **53(1):** 100-111.
Nowak G., Megyesi J., Craigen W.J. (2020). Deletion of VDAC1 hinders recovery of mitochondrial and renal functions after acute kidney injury. *Biomolecules*, **10(4):** 585.
Pennock R., Bray E., Pryor P., James S., McKeegan P., Sturmey R., Genever P. (2015) Human cell dedifferentiation in mesenchymal condensates through controlled autophagy. *Sci. Rep*., **5:** 13113.
Perry H.M., Huang L., Wilson R.J. et al. (2018) Dynamin-related protein 1 deficiency promotes recovery from AKI. *J. Am. Soc. Nephrol*., **29(1):** 194-206.
Quan Y., Xin Y., Tian G., Zhou J., Liu X. (2020) Mitochondrial ROS-modulated mtDNA: A potential target for cardiac aging. *Oxidative Medicine and Cellular Longevity*, **2020:** 9423593.
Quirós P.M., Mottis A., Auwerx J. (2016) Mitonuclear communication in homeostasis and stress. *Nat. Rev. Mol. Cell Biol*., **17(4):** 213-226.
See E.J., Jayasinghe K., Glassford N. et al. (2019) Long-term risk of adverse outcomes after acute kidney injury: a systematic review and meta-analysis of cohort studies using consensus definitions of exposure. *Kidney Int*., **95(1):** 160-172.
Sharifi-Rad M., Anil Kumar N.V., Zucca P. et al. (22 authors) (2020) Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology*, **11(694).**
Suliman H.B., Piantadosi C.A. (2016) Mitochondrial quality control as a therapeutic target. *Pharmacol. Rev*., **68(1):** 20-48.
Szeto H.H. (2017) Pharmacologic approaches to improve mitochondrial function in AKI and CKD. *J. Am. Soc. Nephrol.*, **28(10):** 2856-2865.
Tábara L.C., Poveda J., Martin-Cleary C., Selgas R., Ortiz A., Sanchez-Niño M.D. (2014)

Mitochondria-targeted therapies for acute kidney injury. *Expert. Rev. Mol. Med*., **16:** e13.

Thomasova D., Anders H.-J. (2014) Cell cycle control in the kidney. *Nephrology Dialysis Transplantation*, **30(10):** 1622-1630.

Tran M.T., Zsengeller Z.K., Berg A.H. et al. (11 authors) (2016) PGC1α drives NAD biosynthesis linking oxidative metabolism to renal protection. *Nature*, **531(7595):** 528-532.

Turrens J.F. (2003) Mitochondrial formation of reactive oxygen species. *J. Physiol*., **552(Pt 2):** 335-344.

Valko M., Leibfritz D., Moncol J., Cronin M. T., Mazur M., Telser J. (2007) Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell. Biol.*, **39(1):** 44-84.

Wan J., Kalpage H. A., Vaishnav A. et al (15 authors) (2019) Regulation of respiration and apoptosis by cytochrome c threonine 58 phosphorylation. *Scientific Reports*, **9(1):** 15815.

Wang C., Youle R.J. (2009) The role of mitochondria in apoptosis*. *Annu. Rev. Genet*., **43:** 95-118.

Wang Z., Fan M., Candas D. et al. (19 authors) (2014) Cyclin B1/Cdk1 coordinates mitochondrial respiration for cell-cycle G2/M progression. *Developmental Cell*, **29(2):** 217-232.

Weidemann M.J., Krebs H.A. (1969) The fuel of respiration of rat kidney cortex. *Biochem. J.*, **112(2):** 149-166.

West A.P., Shadel G.S., Ghosh S. (2011) Mitochondria in innate immune responses. Nat Rev Immunol **11**(6): 389-402.

Wu C.K., Wu C.L., Su T.C. et al. (2019) Renal tubular TRPA1 as a risk factor for recovery of renal function from acute tubular necrosis. *J. Clin. Med.*, **8(12).**

Xia M., Zhang Y., Jin K., Lu Z., Zeng Z., Xiong W. (2019) Communication between mitochondria and other organelles: a brand-new perspective on mitochondria in cancer. *Cell Biosci.*, **9:** 27.

Xiao L., Xu X., Zhang F. et al. (16 authors) (2017) The mitochondria-targeted antioxidant MitoQ ameliorated tubular injury mediated by mitophagy in diabetic kidney disease via Nrf2/PINK1. *Redox Biol*., **11:** 297-311.

Zhan M., Brooks C., Liu F., Sun L., Dong Z. (2013) Mitochondrial dynamics: regulatory mechanisms and emerging role in renal pathophysiology. *Kidney Int*., **83(4):** 568-581.

Zhu J., Zhang S., Geng Y., Song Y. (2018) Transient receptor potential ankyrin 1 protects against sepsis-induced kidney injury by modulating mitochondrial biogenesis and mitophagy. *Am. J. Transl. Res.*, **10(12):** 4163-4172.

Zoja C., Benigni A., Remuzzi G. (2014) The Nrf2 pathway in the progression of renal disease. *Nephrol. Dial. Transplant*., **29 (Suppl 1):** i19-i24.

Böyrək zədələnməsində mitoxondrial disfunksiya: potensial terapevtik yanaşmalar

[1,2] Əhmədian Elham Əsgər oğlu, [2,3,4] Xəlilov Rövşən İbrahimxəlil oğlu,
[2,5] Eftexari Əziz Məhəmməd oğlu

[1] *Böyrək Tədqiqat Mərkəzi, Təbriz Tibb Elmləri Universiteti, Təbriz, İran*
[2] *Nanobiotexnologiya və Funksional Nanosistemlər üzrə Ukrayna-Azərbaycan Birgə Beynəlxalq Tədqiqat və Tədris Mərkəzi, Drohobiç, Ukrayna və Azərbaycan*
[3] *Biofizika və biokimya kafedrası, Bakı Dövlət Universiteti, Bakı, Azərbaycan*
[4] *Radiasiya Problemləri İnstitutu, Azərbaycan Milli Elmlər Akademiyası, Bakı, Azərbaycan*
[5] *Farmakologiya və toksikologiya kafedrası, Təbriz Tibb Elmləri Universiteti, Təbriz, İran*

Mitoxondriyalar ATF sintezi, kalsium homeostazı, hüceyrənin sağ qalması və hüceyrə ölümü daxil olmaqla müxtəlif hüceyrə funksiyaları üçün vacib olan unikal orqanellalardır. Mitoxondriya eukariotik hüceyrələrdə mühüm enerji istehsalı mənbəyi olmaqla, həmçinin lipidlər, nuklein turşuları və amin turşularının sintezində də əhəmiyyətli rol oynayır. Mitoxondriya oksidləşdirici stresə həssasdır. Hüceyrələrdə oksigenin reaktiv formalarının (ORF) sintezinin əsas mənbələri mitoxondriya və NADPH oksidazadır (NOX). Mitoxondriyada ORF tənəffüs zəncirində, NOX vasitəsilə isə neytrofillərin və faqosomların membranları boyunca istehsal olunur. Müəyyən şərtlər altında OH●- və O_2●- kimi sərbəst radikalların istehsalı zəifliklərlə nəticələnir. Mitoxondriya tərəfindən istehsal olunan ORF, histonların olmaması səbəbindən oksidləşdirici stress üçün həssas bir hədəfə çevrilən lipidlər, zülallar, DNT, RNT və mitoxondrial DNT (mtDNT) daxil olmaqla bir çox molekullara təsir edir. Zədələnmiş mtDNT və pozulmuş mitoxondrial genom bütövlüyü qocalma ilə əlaqəli xroniki və ağır erkən xəstəliklərin inkişafında əsas rola malikdir. Getdikcə daha aydın olur ki, mtDNT-nin uzunmüddətli kiçik zədələnməsi təkcə qocalma prosesi ilə deyil, həm də diabet və nefropatiyalarla sıx əlaqəli ola bilər. Böyrək xəstəliklərində mitoxondrial disfunksiya əsas rol oynayır. mtDNT, ORF və iltihab faktorları arasında epigenetik dəyişikliklər və qarşılıqlı təsirlər nefronlara təsir göstərir. mtDNT-dəki dəyişikliklər xroniki böyrək xəstəliyinin yaranmasına və inkişafına təsir göstərir. mtDNT-nin dəyişikliyi həmçinin nefropatiyaların monitorinqində də əhəmiyyətli rola malikdir. Sübut olunub ki, qan dövranında və sidikdə mtDNT-nin bir neçə nüsxəsinin modifikasiyasının aşkarlanması mitoxondrial disfunksiyanı və böyrək xəstəliyinin şiddətini əks etdirir. Bu məqalədə nefropatiyaların müalicəsində mitoxondrial antioksidantlar haqqında məlumat verilir. Məqsədli mitoxondrial antioksidantlardan istifadə nefropatiyaların müalicəsində yeni bir fikir olacaq. mtDNT də terapevtik hədəf ola bilər.

Keywords: *mitoxondrial; böyrək xəstəliyi; mtDNT; oksidləşdirici stres*

Митохондриальная дисфункция при повреждении почек: потенциальные терапевтические подходы

[1,2] Ахмадиан Эльхам Аскер оглу, [2,3,4] Халилов Ровшан Ибрагимхалил оглу, [2,5] Эфтехари Азиз Магомед оглу

[1]Почечный исследовательский центр Тебризского университета медицинских наук, Тебриз, Иран
[2]Совместный Украинско-Азербайджанский международный научно-образовательный центр нанобиотехнологий и функциональных наносистем, Дрогобич, Украина и Азербайджан
[3]Кафедра биофизики и биохимии Бакинского государственного университета, Баку, Азербайджан
[4]Институт радиационных проблем НАН Азербайджана, Баку, Азербайджан
[5]Кафедра фармакологии и токсикологии Тебризского университета медицинских наук, Тебриз, Иран

Митохондрии являются уникальными органеллами, которые выполняют различные клеточные функции, включая синтез АТФ, гомеостаз кальция, выживание клеток и их гибель. Митохондрии являются важным источником производства энергии в эукариотических клетках, а также играют существенную роль в синтезе липидов, нуклеиновых кислот и аминокислот. Митохондрии чувствительны к окислительному стрессу. Основными источниками синтеза АФК в клетках являются митохондрии и НАДФН-оксидаза (NOX). В митохондриях АФК продуцируются в дыхательной цепи, а посредством NOX – вдоль мембран нейтрофилов и фагосом. При определенных условиях производство свободных радикалов, таких как OH●- и O_2●-, приводит к уязвимости. АФК, продуцируемые митохондриями, вследствие отсутствия гистонов имеют множество уязвимых для окислительного стресса мишеней, включая липиды, белки, ДНК, РНК и митохондриальную ДНК (мтДНК). Нарушения мтДНК и целостности митохондриального генома играют главную роль в развитии тяжелых ранних и хронических заболеваний, связанных со старением. Становится все более очевидным, что долгосрочные крошечные повреждения мтДНК связаны не только с процессом старения, но также могут быть тесно связаны с диабетом и нефропатиями. Митохондриальная дисфункция играет основную роль в развитии почечных заболеваний. Эпигенетические изменения и взаимодействия между мтДНК, АФК и воспалительными факторами влияют на нефроны. Изменения мтДНК влияют на развитие и прогрессирование хронической болезни почек. Изменение мтДНК также играет важную роль в мониторинге нефропатий. Имеющиеся данные свидетельствуют о том, что модификация нескольких копий мтДНК в кровотоке и моче отражает дисфункцию митохондрий и тяжесть заболевания почек. В этом обзоре описаны митохондриальные антиоксиданты, используемые в терапии нефропатий. Целевые митохондриальные антиоксиданты станут новой точкой зрения в терапии нефропатий. мтДНК также может выступать в качестве терапевтической мишени.

Ключевые слова: *Митохондриальный, почечная болезнь, мтДН, окислительный стресс*

Journal of Life Sciences & Biomedicine, vol. 4(77), No 1, p. 68-75 (2022)

Flora of the surrounding of the Yenikend reservoir and its analysis

***Ibadullayeva Sayyara Jamshid, Sadigova Narmin Ilham**

Institute of Botany, Azerbaijan National Academy of Sciences, 40 Badamdar Ave., Baku AZ1004, Azerbaijan
****For correspondence:** ibadullayeva.sayyara@mail.ru*

Received: March 02, 2022; Reviewed: April 30, 2021; Accepted: June 01, 2022

The article is dedicated to the study of the surrounding flora of the Yenikend reservoir in the Samukh district of the Lesser Caucasus. During the study, in the area, 594 species belonging to 81 families and 316 genera were determined, a new habitat of 4 species was discovered, and 3 species were already considered extinct in the area. In the flora of the area, angiosperms belonging to 14 families (between 5-26 genera each) are dominated by 187 genera and 393 species. Each of the remaining 68 families is represented by only 1-4 genera. According to biomorphological composition, its predominance with 273 species was determined. There are trees with 28 species, shrubs with 55 species, subshrubs with 7 species, and small shrubs with 11 species. More than half of the area flora is occupied by hemicryptophytes (267 species), followed by terophytes (190 species). Ecological analysis of the area of the Yenikend reservoir showed that some xerophytes are also resistant to moisture due to the rising and falling water level. In terms of number, they occupy the main place in the area with 283 species, mesoxerophytes with 117 species make up 19.7%, and xeromesophytes with 92 species make up 15.5% of the total flora. The species in the area were geographically analyzed and 17 classes and 82 groups included in 7 genetic (ancient, boreal, steppe, xerophile, desert, Caucasian, adventive) and 5 zonal (nemoral, arid, arcto-alpine, montana, cosmopolite) types of habitats were identified. The similarity coefficient of the floristic composition by the zones was calculated using the Sorensen-Czekanowski similarity coefficient (K_{sc}). The coefficient of similarity of the subalpine compared to high-mountains, the plains to the mid-mountains, the low-mountains to the mid-mountains is K_{sc}=0.45; 0.39; 0.37.

***Keywords**: Yenikend, reservoir, flora, taxon, geographical area*

INTRODUCTION

The study and protection of the modern state of flora and vegetation of Azerbaijan are at the center of attention with its relevance. The study of the modern flora and vegetation of specific areas, identifying the changes that have taken place there, and conducting research against environmental, anthropogenic and zoogenic impacts are of great theoretical and practical importance. One of such specific areas surrounded by the flora of the Lesser Caucasus is the surrounding area of the Yenikend reservoir located in the Samukh district. The area is connected with the Kura River, Ganjachay and Goshgarchay valleys. Frequent rises and falls of water and other anthropogenic impacts here led to washing of the slopes, an increase in erosion processes and changes in the composition, structure and productivity of flora and vegetation. As a result, forests and shrubs were gradually reduced, many of which were completely destroyed, and invasive plants formed the jungle in their place. Therefore, the restoration of eroded areas of the water basin on a scientific basis is considered an urgent task.

A number of prominent scientists conducted research on the flora, vegetation of various river basins and water reservoirs as well as their phytomeliorative importance: S.V.Gudoshnikov (1953), A.M.Moldoyarov (1964), R.V.Kamelin (1973), M.Darvoziyev (1977), in Azerbaijan A.G.Dolukhanov (1969), V.J.Hajiyev (1965), E.M.Gurbanov (1996), A.H.Ismayilov (2009), etc. Expedition routes to the Lesser Caucasus by

10.5281/zenodo.7239696
Available online 30 June 2022

V.J.Hajiyev (1990) conducted in 1986-2004 covered certain areas and the plant and geological materials were collected. The studies were also conducted at the border of the Samukh district, but the surrounding area of the Yenikend reservoir has been never studied as a separate research object. Taking into account all this, both the district and surrounding area of the reservoir were geobotanically inspected.

The main purpose of the research is to study the modern flora of the Yenikend reservoir surroundings. For this purpose, the following specific tasks were set: to give a complete description of the flora of the study area based on its ecobiomorphological and geographical analysis, to identify endemic, relict, rare and endangered species.

MATERIALS AND METHODS

The research was carried out in the area flora of the Yenikend reservoir located in the Samukh district of the Lesser Caucasus in 2015-2021. For this purpose, short and long-term expeditions to the basin area on 25 routes were carried out annually in spring, summer and autumn, and more than 600 herbarium materials were collected. The studies were carried out by semi-stationary and stationary methods, more than 50 geobotanical notes on the structure of phytocoenoses were taken, photos of rare species and formations were taken separately. Although the identification of species was based on the fundamental flora of the former USSR, Caucasus, and Azerbaijan, the taxa were mainly called according to the "Conspectus of the flora of Caucasus" (2006). Classical and modern - botanical, floristic, systematic, areological, ecological methods were used in the development of materials (Shennikov, 1964; Rabotnov, 1950, etc.). During the expeditions, the eco-biological characteristics of the species were clarified based on the generally accepted methodology of geobotanical search in the field, and botanical descriptions and phenological observations were made (1966). Analysis of the main life forms of plants in the flora of the study area was carried out based on the systems of I.G.Serebryakov (1964) and C.Raunkiaer (1934). The degree of similarity of the taxonomic composition comparable to the zones was calculated using the Sorensen-Czekanowski formula. $Ksc=2c/a+b$ Here: a-indicates the number of species in one zone, b-the number of species in the other zone, c-the number of species common to both zones (1961).

RESULTS AND DISCUSSION

The study area is located at the north-western end of the Republic of Azerbaijan and extends from the north-west to the south-east. The study areas where wastewater is discharged are mainly located on the plains on the right and left banks of the Kura River. The Gabirri and Ganikh rivers pass nearby, and the Gushgara river flows through the Garayeri settlement.

The area borders Georgia to the north and northwest, and the middle courses of the Kura River to the southwest and south. Its area is 1450 km^2, of which 53500 ha (22.1%) is used as winter pasture. The absolute altitude of the area varies between 220-871 m. It belongs to the Samukh district by administrative division. The structure of the area is complex in appearance and is characterized by a combination of micro, meso and macrorelief types. The main part is located in the plain, and there are no very high hills. Although the slope of the area is from south to north, it was observed that in some places the direction of the slope is from north-east to south-west, from east to west and from west to east.

On a sloping plain stretching along the Kura River from west to east, land resources are mainly used as winter pastures. In the foothill plain, land resources used mainly in agriculture are distributed. In the mid-mountain belt with a fragmented surface structure, there are lands that partially meet the needs of agriculture and mainly livestock, as well as forest fund lands. Land resources used as summer pastures are spread in the high mountain belt.

As a result of previous studies, the plants distributed in the flora of the area were systematically (Table 1) and taxonomically analyzed. During the study of the surrounding flora of the Yenikend reservoir, it was revealed that there are 594 species belonging to 81 families and 316 genera. New habitat of 4 species (*Cyperus rotundus* L., *Carex canescens* L. (=*Carex cinerea* Poll.), *Bryona alba* L., *Luzila multiflora* Ehrh.Lej.) was found, 3 species (*Buglossides tenuiflora* (L.f.) I.M.Johnst., *Gypsophila muralis* L., *Astragalus dzhebrailicus* Grossh.) were already considered extinct in the area.

Representatives of angiosperms (magnolia) belonging to 14 families (between 5-26 genera each) in the area flora are dominated by 187 genera (59.4%) and 393 (66.5%) species. Each of the remaining 68 (83%) families was represented by only 1-4 species (Fig. 1).

As can be seen from the floristic spectrum of the area, each of the 13 families is represented by 5 or more genera. The remaining 68 families were represented by 4 or fewer species. However, among them, there are genera that are important in the phytocoenosis with more species than others (Fig. 2).

Table 1. Systematic structure of the area flora

№	Division and classes of plants	Families		Genera		Species	
		Number	By %	Number	By %	Number	By %
1	*Equisetophyta*	1	1.21	1	0.36	3	0.50
2	*Polypodiphyta*	4	4.87	6	7.90	9	1.52
3	*Pinophyta*	2	2.43	2	0.63	5	0.84
4	*Gnetophyta*	1	1.21	1	0.31	1	0.16
5	*Magnoliophyta*	74	90.2	305	96.82	573	96.95
a	*Magnoliopsida*	62	75.6	254	80.63	469	79.35
b	*Liliopsyda*	12	14.63	51	16.19	104	17.59
Total		82	100	315	100	591	100

The number of genera with many species is much higher than the averagenumber. Each of the 13 genera in the area flora has 5 or more species, and each of the 4 genera has 10 or more species, which are polymorphic plants. Biomorphological analyzes were conducted and grasses with 273 species were found to be predominant (Table 2, Table 3).

More than half of the flora of the area is occupied by hemicryptophytes, followed by therophytes. Epiliths and epigeia which are typical for life forms of mosses are additionally included in the classification.

During the analysis of the area flora by ecological groups, life conditions of plants with different degrees of humidity were considered (Table 4). Analysis of the studied plants by ecological groups shows that the humidity degree of the plants varies. Water is one of the most important environmental factors in plant life. Adaptation of plants to habitats with different degrees of humidity, adaptation traits acquired in connection with adaptation divides them into ecological groups that are different from each other.

Table 2. Life forms of area plants according to Serebryakov (1964)

№	Life forms	Species	
		Number	By total number, in%
1	Trees	28	4,7
2	Tree and shrubs	5	0,84
3	Shrubs	55	9,25
4	Subshrubs	7	1,17
5	Small shrubs	11	1,81
6	Grasses: including a) perennial b) biennial c) annual e)annual and biennials	273 30 172 15	45,95 5,1 28,9 2,52
Total:		594	100

Table3. Life forms of plants of the area flora according to Raunkier (1934)

№	Biomorphological groups	Species	
		Number	By total number, in%
1	Phanerophytes	90	15,15
2	Chamephytes	16	2,69
3	Hemicryptophytes	267	44,94
4	Cryptophytes Including: a) bulbous geophytes b) rhizomatous c) root tubers	31 20 4 7	5,24
5	Therophytes	190	31,98
Total:		594	100

Water has a great influence on the distribution of plants in small areas and grouping in certain areas under different climatic conditions or in the same climatic conditions. The water demand of plants living in a xerophytic environment is conditionally due to two reasons: reaching of water to the soil is limited due to low rainfall, the amount of water consumed by the plant as a result of transpiration is greater than the amount of water the plant receives from the soil.

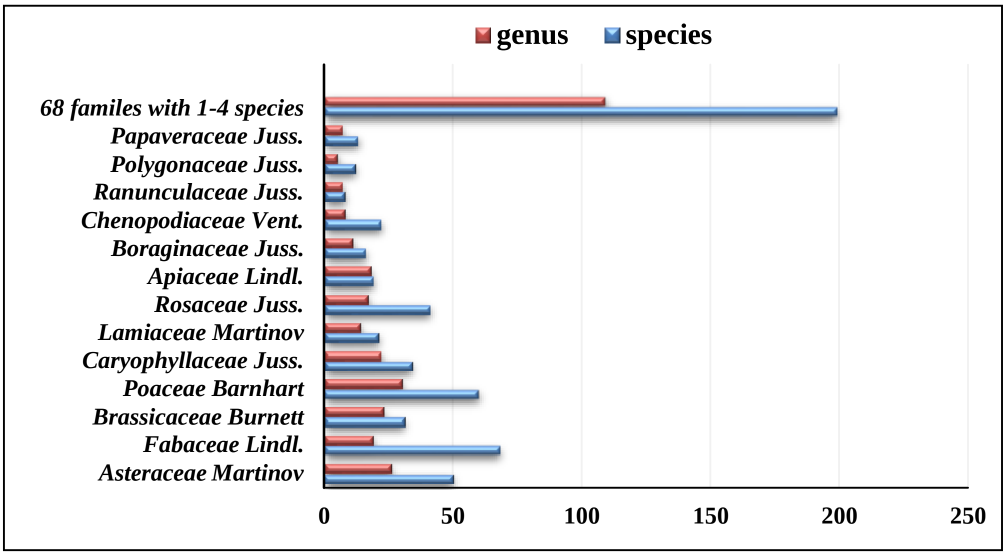


Fig. 1. Taxonomic composition of the surrounding flora of Yenikend reservoir.

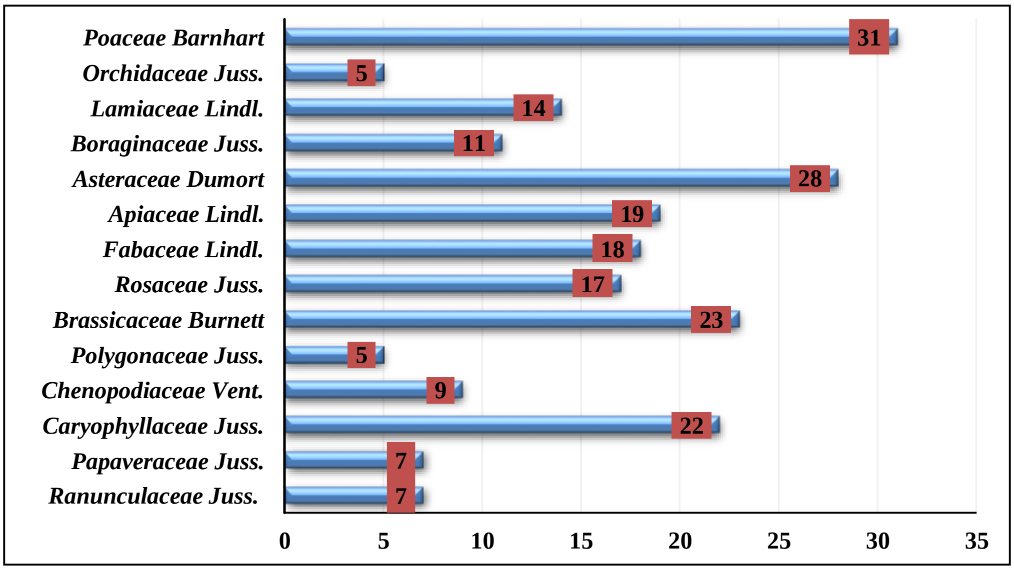


Fig. 2. Genera represented by more species in the area flora.

Due to the lack of humidity in plant habitat, its periodic low or high level and increase in physiological resistance, plants occupy an intermediate position between mesophytes and xerophytes and are called mesoxerophyte and xeromesophyte plants. By origin, mesophytes are called mesoxerophytes when they adapt to the environment and lead a relatively xerophytic life, called a xeromesophyte if it adapts to a xerophic environment and leads a relatively mesophytic life.

Ecological analysis of the area of the Yenikend reservoir showed that some xerophytes are also resistant to moisture due to rising and falling of water: mesoxerophytes with 117 species make up 19.7%, and xeromesophytes with 92 species make up 15.5% of the total flora. Thus, in terms of number, xerophytic plants occupy the main place in the area with 283 species (47.9%). Xerophytes are plants of arid areas and adapt well to water shortages.

Table 4. Ecological groups of area plants according to Shennikov (1964)

No	Ecological groups	Species	
		Number	By total number, in%
1.	Xerophytes	281	47.3
2.	Mesoxerophyte	117	19.69
3.	Xeromesophyte	92	15.84
4.	Mesophyte	96	16.16
5.	Hygrophyte	5	0.84
6.	Hydrophyte	1	0.16
Total:		594	100

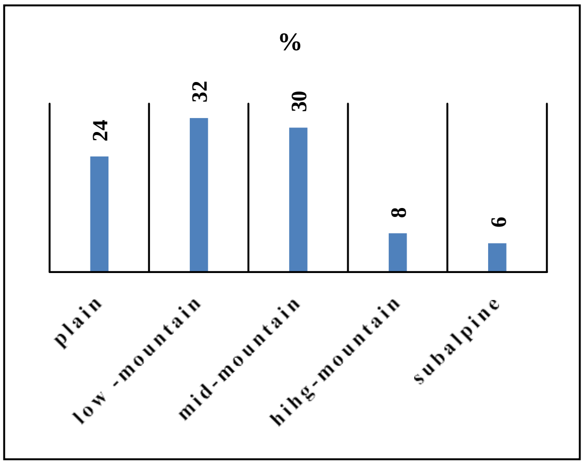


Fig. 3. Distribution of plants by altitude zones

Table 5. Coefficient of similarity to the floristic composition compared to the zones

Zones	Plain	Low- mountain	Mid-mountain	High-mountain	Subalpine
Plain	-	1.93	1.25	1.71	0.125
Low- mountain	-	-	1.06	1.62	1.71
Mid-mountain	-	-	-	1.9	0.19
High-mountain	-	-	-	-	0.34
Subalpine	-	-	-	-	-

Xerophytes, mesoxerophytes and mesophytes play an important role in the development of modern flora of the area.

Paying particular attention to species that cross the boundaries of their range, the study of geographical and genetic elements of flora, the central issues of the origin of individual plants are based on the systems of A.A.Grossheim and N.N.Portenier (which is developed for vascular plants of the Caucasus region). During the study of the area flora, the species in the area flora were geographically analyzed based on the geographical types of A.A.Grossheim, a great researcher of the Caucasian flora and 17 classes and 82 groups including in 7 genetic (ancient, boreal, steppe, xerophile, desert, Caucasian, adventive) and 5 zonal (nemoral, arid, arcto-alpine, montana, cosmopolite) types of habitats were identified.

An analysis of early spring flora was made and it was found that 84 species of plants belonging to 31 families were spread in the early spring flora of the area, of which 42 were ephemeral and 22 were ephemeroids.

Distribution patterns of plants in altitude zones were analyzed. When determining the flora and vegetation of the area by altitudes, the regularity of the distribution of the species on taken four vertical belts was studied. The number of species in the lower zone of the area is higher than in other zones (Fig. 3, Table 5). A comparison of the floristic composition of the zones was calculated using the Sorensen-Czekanowski similarity coefficient (K_{sc}). The coefficient of similarity of the subalpine compared to the highlands, the plains to the mid-mountains, the low mountains to the mid-mountains is K_{sc}=0.45; 0.39; 0.37.

REFERENCES

Darvoziev M. (1977) Flora and vegetation of the Yazgulem river basin. *Abstract diss cand. biol. Sciences*. Dushanbe: 19 p. *(in Russian)* (**Дарвозиев М.** Флора и растительность басейна реки Язгулем. *Автореф. дисс канд. биол. наук.* Душанбе: 19 с.)

Dolukhanov A.G. (1969) On some features of rock-scree high-mountain vegetation in the upper reaches of the large liati. *Bulletin of the Moscow Society of Naturalists*, **No 6:** 86-93 (in Russian) (**Долуханов А.Г.** (1969) О некоторых особенностях скально-осыпной высокогорной растительности в верховьях большой лиати. *Бюлл. Моск. Общ-воисп. Природы,* **вып. 6:** *86-93)*

Flora of Azerbaijan (1950-1961) In 8 vols. V. 1-8, Baku: Pub. AS of Azerbaijan SSR, (in Russian). (**Флора Азербайджана** (1950-1961) В 8-х т. т. 1-8, Баку: Изд. АНАзерб. ССР.)

Gadzhiev V.D. (1965) Materials for the study of vegetation and phytomelioration of the river basin of Kur-Mukhchay. The News of AS Azerb. SSR, series of biol. sciences, **No 2:** 15-21 (in Russian) (**Гаджиев В.Д.** (1965) Материалы к изучению растительности и фитомелиорации бассейна р. Кур-Мухчай. *Из-в. АН Азерб. ССР, серия биол. наук,* **№2:** 15-21.)

Gadzhiev V.D., Aliyev D.A., Kuliyev V.Sh. et al. (1990) High-mountain vegetation of the Lesser Caucasus. Baku: Elm, 212 p. (in Russian) (**Гаджиев, В.Д., Алиев Д.А., Кулиев В.Ш. и др.** (1990) Высокогорная растительность Малого Кавказа. Баку: Элм, 212 с.)

Grossheim A.A. (1936) Analysis of the flora of the Caucasus. *Pr.of Inst.Bot. Az. BAS USSR* (Baku), **1:** 260 p. (in Russian) (Гроссгейм А.А. (1936) Анализ флоры Кавказа. *Тр. Бот. Ин-та Аз. ФАН СССР* (Баку), **1:** 260 с.)

Gudoshnikov S.V. (1953)The vegetation of the river basin Djidi within the Buryat-Mongolian ASSR: *Abstract of the dis. ... cand. biol. Sciences*. Tomsk: 13 p. (in Russian) (**Гудошников С.В.** (1953) Растительность бассейна р. Джиды пределах Бурят-Монгольской АССР: *Автореф. дис канд. биол. наук.* Томск: 13 с.).

Gurbanov E.M. (1996) Flora of the river basin. River Nakhchivanchay. Baku: p. 51-57. (in Russian) (**Гурбанов Э.М.** (1996) Растительный мир бассейна р. Нахчеванчай. Баку: с. 51-57.)

Ismayilov A.H. (2009) Flora, vegetation of the Gilanchay basin in the Nakhchivan Autonomous Republic and their phytomeliorative significance: *Abstr. of diss. ... Doctor of Philosophy in Biological Sciences*. Baku: 22 p. (in Azerbaijan) (**İsmayılov A.H.** (2009) Naxçıvan Muxtar Respublikasında Gilançay hövzəsinin florası, bitkiliyi və onların fitomeliorativ əhəmiyyəti: *Biol. elm. üzrə fəls. dok dis. avtoref*. Bakı: 22 s.)

Kamelin R.V. (1973) Floristic analysis of the natural flora ofmountainous Central Asia. L.: Nauka, 355 p. (in Russian) (**Камелин Р.В.** (1973) Флористический анализ естественной флоры горной Средней Азии. Л.: Наука, 355 с.)

Karyagin I.I. (1936) New species for the flora of the Caucasus. *Pr.of Inst. Bot. Az. BAS USSR*, **2:** 263-267 (in Russian) (**Карягин И.И.** (1936) Новые виды для флоры Кавказа. *Тр. АзФАН СССР,* **2:** 263-267).

Method of phenological observations in botanical research (1966) M.-L.: Nauka, 152 p. (in Russian) (**Метод фенологических наблюдений при ботанических исследованиях** (1966) М.-Л.: Наука, 152 с.)

Methods for isolating plant associations (1971) Man. ed. V.D.Aleksandrova. L.: Nauka, p. 63-70 (in Russian) (Методы вделения растительных ассоциаций (1971) Отв. ред. В.Д.Александрова. Л.: Наука, с. 63-70).

Methods of field geobotanical research (1938) M., L.: AS USSR, 214 p. (in Russian) (**Методика полевых геоботанический исследований** (1938) М., Л.: АН СССР, 214 с.)

Rabotnov T.A. (1950) Life cycle of perennial herbaceous plants of meadow cenoses. *Pr. Inst.of Bot. of the AS of the USSR, ser. geobotany*, **No 6:** 204 p. (in Russian) (**Работнов Т.А.** (1950) Жизненный цикл многолетних травянистых растений луговых ченозов. *Тр. Ботан. ин-та АН СССР, сер. геоботаники,* **вып. 6:** 204 с.)

Raunkaier C. (1934) The life form of plants and statistical plantgeoqraphy. Oxford: Clparendon Press, p. 48-154.

Serebryakov I.G. (1962) Life forms of higher plants and their study. *Field Geobotany,* In 3 vols. M.-L.: Pub. House Academy of Sciences of the USSR, **3:** 181 p. (in Russian) (**Серебряков И.Г.** (1962) Жизненные формы высших растений и их изучение. Полевая геоботаника, В 3-х т. М.-Л.: Изд. АНСССР, **3:** 181 с.).

Shennikov A.P. (1964) Introduction to geobotany. Leningrad: Pub. Lening. Un-t, 447 p. (in Russian) (**Шенников А.П.** (1964) Введение в геоботанику. Ленинград: Из-во. Ленингр. Ун-та, 447 с.)

Synopsis of the flora of the Caucasus (2006) In 3 vols. Under. Ed. Yu.L.Menitsky, T.N.Popov. St. Petersburg Publishing House, **2:** 466 p. (in Russian) **(Конспект флоры Кавказа** (2006) В 3-х т. Под. Ред. Ю.Л.Меницкий, Т.Н.Попова. СПб.: С.-Петерб. ун-т, **2:** 466 с.)

Yaroshenko P.D. (1961) Geobotany (basic concepts, directions and methods). L.: Publishing House of the Academy of Sciences of the USSR, 474 p. (in Russian) (**Ярошенко П.Д.** (1961) Геоботаника (основные понятия, направления и методы). Л.: АН СССР, 474 с.

Yenikənd su anbarı ətrafının florası və onun təhlılı

Ibadullayeva Səyyarə Cəmşid qızı, Sadıqova Nərmin İlham qızı

AMEA-nın Botanika İnstitutu, Bakı, Azərbaycan

Məqalə Kiçik Qafqazın Samux rayonu ərazisində Yenikənd su anbarının ətraf florasının öyrənilməsinə həsr edilmişdir. Tədqiqat zamanı ərazi florasından 81 fəilə, 316 cinsə aid 594 növün olması müəyyən edilmiş, 4 növün yeni arealı aşkar edilmiş, 3 növ isə artıq ərazidə itmiş hesab edilmişdir. Ərazi florasında 14 fəsiləyə aid örtülütoxumlular (hərəsi 5-26 cins arasında) 187 cins və 393 növlə üstünlük təşkil edirlər. Qalan 68 fəsilənin hər biri cəmi 1-4 cinslə təmsil olunmuşdur. Biomorfoloji tərkibə görə 273 növlə üstün olması müəyyən edilmişdir, ağaclar 28, kollar 55, yarımkollar 7, kolcuqlar 11 növlə rast gəlinir. Ərazi florasının yarıdan çoxunu hemikriptofitlər (267 növ), ikinci yeri isə terofitlər (190 növ) tutur. Yeni Kənd su anbarı ərazisinin ekoloji təhlili göstərdiki suyun qalqıb-enməsilə bağlı bəzi kserofitlər də rütubətə davamlılıq göstərirlər. Onlar ərazidə say tərkibinə görə 283 növü əhatə etməklə əsas yeri tutur, mezokserofitlər 117 növlə ümumi floranın 19,7%-ni, kseromezofitlər isə 92 növ olmaqla 15,5 % təşkil edir. Ərazi florasındakı növlər coğrafi təhlil edilməklə, arealın 7 genetik (qədim, boreal, bozqır, kserofil, səhra, qafqaz, adventiv), 5 zonal (nemoral, arid, arkto-alp, montan, kosmopolit) tipinə daxil olan 17 sinif və 82 qrupu müəyyən olunmuşdur. Bitkilərin qurşaqlara görə paylanması floristik tərkibinin müqayisəsi Serensen-Çekanovski oxşarlıq əmsalından istifadə etməklə hesablanmışdır: subalpın yüksəkdağlığa, düzənliyin orta dağlıqla, aşağıdağlığın orta dağlıqla müqayisəsində oxşarlıq əmsalı K_{sc}=0,45; 0,39; 0,37 .

Açar sözlər: *Yenikənd, su anbarı, flora, takson, coğrafi areal*

Флора вокруг водохранилища Йеникенд и ее анализ

Ибадуллаева Сайяра Джамшид кызы, Садыгова Нармин Ильхам кызы

Институт ботаники НАН Азербайджана, Баку, Азербайджан

Статья посвящена изучению флоры вокруг водохранилища Йеникенд в Самухском районе Малого Кавказа. В ходе исследований установлено, что во флоре района насчитывается 81 семейство, к которым относятся 594 вида 316-ти родов. Обнаружены новые ареалы 4 видов, 3 из которых считаются исчезнувшими для данной территории. Во флоре территории преобладают Покрытосеменные с 14 семействами (от 5 до 26 видов в каждом), 187 родами и 393 видами. Каждое из оставшихся 68 семейств представлено только 1-4 родами. По биоморфологическому составу преобладают 273 вида,

28 видов деревьев, 55 видов кустов, 7 видов полукустарников и 11 видов кустарничков. Более половины флоры района представлены гемикриптофитами (267 видов), за ними следуют терофиты (190 видов). Экологический анализ территории водохранилища Йени йол показал, что при притоке и оттоке воды в водохранилище некоторые ксерофиты также проявляют устойчивость к влажности. Они, с численностью 283 вида, занимают основное место, мезоксерофиты со 117 видами составляют 19,7%, а ксеромезофиты с 92 видами составляют 15,5% всей флоры исследуемой территории. При географическом анализе видов во флоре района выделено 7 генетических (древние, бореальные, степные, ксерофильные, пустынные, кавказские, адвентивные), 5 зональных типов (неморальные, аридные, арктоальпийские, монтанные, космолитные), включающих 17 классов и 82 группы. Распределение растительности по поясам при сравнение флористического состава рассчитан с использованием коэффициента сходства Серенсена-Чекановского: коэффициент схожести Ksc=0,45; 0,39; 0,37 определен при сравнении субальпийской флоры с высокогорной, равнинной со среднегорной, предгорной со среднегорной.

Ключевые слова: *Йеникенд, водохранилище, флора, таксон, географический ареал*

Journal of Life Sciences & Biomedicine, vol. 4(77), No 1, p. 76-83 (2022)

Non-photochemical quenching of chlorophyll fluorescence and its components – recent advances

Zulfugarov Ismayil Sohbat

Institute of Molecular Biology & Biotechnologies, Azerbaijan National Academy of Sciences, 11 Izzat Nabiyev Str., Baku AZ 1073, Azerbaijan
For correspondence: *i.zulfugarov@imbb.science.az*

Received: May 10, 2022; Received in revised form: May 20, 2022; Accepted: June 06, 2022

To protect themselves from fluctuating light environments, plants have evolved non-photochemical quenching (NPQ) as a protective mechanism. NPQ comprises the thermal dissipation of excess light energy via the de-excitation of singlet excited chlorophyll (Chl) in photosystem II of photosynthetic organisms. In this review, all available data on the NPQ and its components have been summarized. NPQ components were primarily distinguished based on the NPQ relaxation and its sensitivity to chemical inhibitors. However, numerous diverse processes contribute to NPQ therefore, it has been suggested to separate NPQ components based on the molecular players involved as well as on Chl fluorescence relaxation kinetics. These types of NPQ include energy-dependent quenching (qE), state transition quenching (qT), photoinhibitory quenching (qI), sustained quenching (qH), zeaxanthin-dependent quenching (qZ), and chloroplast movement-dependent quenching (qM). Although molecular players and regulatory elements that modulate these quenching types are not discussed in this review, they may differ and afford to adapt to the environmental stresses that plants are experiencing. Finally, the role of the investigation of NPQ components, their molecular players, and regulatory mechanisms involved in NPQ as promising targets for strategies to breed highly productive and tolerant crop plants was suggested.

Keywords: *Chlorophyll fluorescence, chloroplast movement-dependent quenching, energy-dependent quenching, non-photochemical quenching, plant, state transition quenching, photoinhibitory quenching, sustained quenching, zeaxanthin-dependent quenching*

INTRODUCTION

A biological process that converts the energy from sunlight into chemical energy is called photosynthesis. In plants, this process assimilates CO_2 and produces biological compounds through the Calvin–Benson–Bassham cycle. Although light is mandatory for photosynthesis, excess light becomes a damaging factor to the photosynthetic apparatus. Usually, the quantity of light changes in natural environments therefore, photosynthetic organisms are often exposed to light stress. When sunlight is in excess, over-excitation of the photosystems occurs and it results in damage to the photosynthetic apparatus. Finally, it may lead to cell death in photosynthesizing organisms. The reaction center of photosystem II (PSII) consumes the energy captured by the antenna and causes a decrease in the density of excited states. This leads to the decrease in the fluorescence intensity/lifetime of the antenna chlorophylls (Chl) and is called photochemical quenching (qP) (Krause and Weis, 1991). Another type of quenching exists when the reaction center of the RCII is closed. This type of quenching mechanism is called non-photochemical quenching (NPQ) of Chl fluorescence (Demmig-Adams et al., 2014). Plants have evolved this NPQ as a protective mechanism that dissipates excess light energy as heat (Müller et al., 2001; Ruban, 2016). NPQ involves the thermal dissipation of excess absorbed energy through the de-excitation of singlet excited Chl in photosystem II (PSII).

10.5281/zenodo.7239759
Available online 30 June 2022

Chl fluorescence is actually an "instrument" that detects the total density of excited states in the photosynthetic antenna (Clayton, 1980; Blankenship, 2002; Ruban, 2012). Thus, NPQ is not a straight measure of dissipated excess energy absorbed by the antenna. Application of photosynthetically active radiance to the photosynthetic organisms drives electron transport following light harvesting by the antenna. Electron transport is associated with the proton gradient across the photosynthetic membrane, ΔpH, which activates NPQ (Briantais et al., 1979; Wraight and Crofts, 1970). Indeed, the acidification of the thylakoid membranes was found to induce the protonation in PSII and trigger the NPQ response (Rees et al., 1992; Liguori et al., 2019; Krishnan-Schmieden et al., 2021; Li et al., 2004). Although the minimum obligation for the NPQ activation is the presence of the LHCII and ΔpH, these factors alone are not enough to induce a significant and fast NPQ response under physiological ΔpH values (Saccon et al., 2020). The xanthophyll cycle and the photosystem II subunit S (PsbS) protein have a decisive role in the NPQ activation, especially under smaller ΔpH values (Johnson and Ruban, 2011). Because plants can alter the gathering of these factors based on metabolic and environmental signals, providing a complex control of the NPQ response. It is important to note that this has a great physiological meaning. It has been shown that NPQ can arise in the absence of PsbS or zeaxanthin, as in wild-type plants, in spite of losing the kinetics of fast activation and deactivation (Johnson and Ruban, 2010; Johnson et al., 2012).

In this review, we summarize all available pieces of information on NPQ and its components because, the rate of induction and relaxation of NPQ components become a leading factor for the growth of plants in the field (Murchie and Niyogi, 2011; Kromdijk et al., 2016). The knowledge about NPQ and its components might be helpful to breed crop plants with high productivity and tolerance to environmental stresses. NPQ components were initially defined based on NPQ relaxation kinetics and its sensitivity to chemical inhibitors (Ruban, 2016; Walters and Horton, 1991). Numerous diverse processes contribute to NPQ. Therefore, Malnoë (Malnoë, 2018) suggests separating them based on the molecular players involved as well as on Chl fluorescence relaxation kinetics. Because of the complexity of the integral components of NPQ and its components, I will not discuss all the players and controllers in this review. These types of NPQ include energy-dependent quenching (qE), state transition quenching (qT), photoinhibitory quenching (qI), sustained quenching (qH), zeaxanthin-dependent quenching (qZ), and chloroplast movement-dependent quenching also called as blue light-dependent quenching (qM) (see Fig. 1).

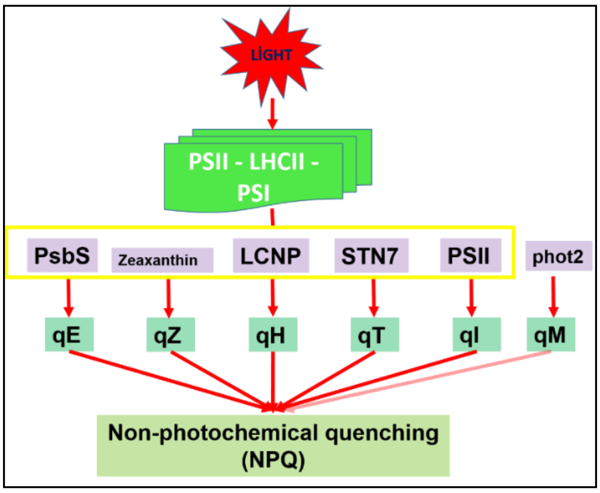


Fig. 1. A schematic diagram illustrating the process of non-photochemical quenching (NPQ) in plants.

Energy-dependent quenching – qE

The fastest and major component of NPQ is the energy-dependent quenching (qE), which is initiated under light and reversed in the dark (Li et al., 2002; Zulfugarov et al., 2007; Ruban et al., 2012; Niyogi and Truong, 2013; Goss and Lepetit, 2015). qE is determined by the acidification of the thylakoid lumen that occurs mainly under high light conditions (Wraight and Crofts, 1970; Briantais et al., 1979). This acidification alters the pigment composition of LHCII primarily through the xanthophyll cycle and triggers qE protein effectors, such as PsbS protein of PSII in plants (Pashayeva et al., 2021)._Evidence collected during the last two decades suggests that the leading quenching site of qE in vivo is the LHCII (Ruban et al., 2012). qE is a ubiquitous energy dissipation mechanism among photosynthetic eukaryotes (Niyogi and Truong, 2013$

Goss and Lepetit, 2015). qE deficient plants become highly sensitive to photoinhibition (Ruban et al., 2012; Niyogi and Truong, 2013; Zulfugarov et al., 2014) and it protects PSII reaction centers against photodamage (Pashayeva et al., 2021; Zulfugarov et al., 2007; Zulfugarov et al., 2014; Zulfugarov et al., 2019). PsbS protein, xanthophyll cycle, and ΔpH co-operate together to induce conformational changes in the thylakoid membrane (Jeong et al., 2010; Zulfugarov et al., 2010). The conformational changes which occur in the thylakoid membranes of plants are proposed to initiate quenching (Ruban et al., 2012). Most importantly, it has been shown that genetic alteration of qE capacity improves crop productivity in the field (Kromdijk et al., 2016).

State transition quenching – qT

The redistribution of excitation energy between photosystems in response to light causes a disproportion in excitation between these photosystems, called state transition (qT) (Bellaflore et al., 2015). State transition limits the over-reduction or over-oxidation of the photosynthetic electron transport chain and also the possibility of photo-oxidative damage (Derks, 2015). Usually, qT occurs under low-light conditions and involves the movement of phosphorylated antenna proteins away from PSII (Krause and Weis, 1991; Ruban and Johnson, 2009). In plants, state transitions occur through LHCII movement in a way different from that used in qE (Pashayeva et al., 2021). qE occurs due to the reversible phosphorylation of the antenna proteins and it regulates the relocation of some populations of LHCII from PSII to PSI.

Photoinhibitory quenching – qI

Photoinhibitory quenching (qI) mainly includes a loss in the number of active PSII reaction centers from photodamage (Derks et al., 2015). Previously the mechanisms that resulted in the light-induced reduction in the quantum yield of PSII because of D1 photoinactivation were termed photoinhibitory quenching (qI) (Krause, 1988; Malnoë, 2018). However, qI is also connected with zeaxanthin maintenance. qI contains all mechanisms with slow relaxation kinetics, such as photoinhibition and other un-considered modes of continuous thermal dissipation (Demmig-Adams and Adams, 1992). qI revenues several hours or longer to relax depending on both photoinhibition and other long-term photoprotective quenching mechanisms (Malnoë et al., 2017; Ruban and Horton, 1995). Thus, qI is the slowest component of NPQ (Zulfugarov et al., 2014).

Sustained quenching – qH

qH is the most newly recognized antenna-quenching component of NPQ, which protects the photosynthetic apparatus under stress conditions in Arabidopsis (Brooks et al., 2013; Malnoë et al., 2017). qH is a different NPQ component independent of PsbS protein of PSII, ΔpH, zeaxanthin, the STN7 kinase, and photoinhibition processes. Because qH is also a slow component of NPQ it is named a sustained quenching that occurs within a photosynthetic antenna (Brooks et al., 2013; Malnoë et al., 2018). This quenching mechanism involves the plastid lipocalin LCNP (Malnoë et al., 2017) and is prevented by the suppressor of quenching1 (SOQ1) under non-stress conditions. The molecular players of qH, LCNP, and the relaxation of qh1 (ROQH1) were suggested to play an antagonistic role in a dosage-dependent manner to protect the photosynthetic apparatus and in sustaining the light-harvesting efficiency in plants (Amstutz et al., 2020).

Zeaxanthin-dependent quenching – qZ

Using different NPQ mutants from Arabidopsis showed that the new type of quenching occurs in plants unrelated to qE, qT, or qI (Nilkens et al., 2010). The analysis of the NPQ induction and relaxation kinetics shows a lifetime in the range of 10–15 min. Because this lifetime correlates with zeaxanthin formation, authors identified this type of quenching as a zeaxanthin-dependent quenching – qZ (Nilkens et al., 2010). qZ is dependent upon the accumulation of zeaxanthin, but its induction is independent of both ΔpH and PsbS (Dall'Osto et al., 2005; Nilkens et al., 2010). qZ is supposed to arise within the minor antennae that persist attached to PSII, which includes minor LHC proteins (Jahns and Holzwarth, 2012). qZ relaxes more slowly than

fastest qE (Dall'Osto et al., 2005; Nilkens et al., 2010; Jahns and Holzwarth, 2012). It has been shown that qZ is the most variable NPQ component in different plants and its variation influenced the strength and the kinetic properties of the response to the given experimental conditions (Sello et al., 2019). Some plants can modulate qZ depending on growth conditions, but probably, this response may also be inclined by species-specific features.

Chloroplast movement-dependent quenching – qM

A blue light-dependent quenching (qM) is tempted by chloroplast movement (Cazzaniga et al., 2013; Dall'Osto et al., 2014). qM is also called chloroplast movement-dependent quenching and it was detected in *phot2* mutant of Arabidopsis with impaired chloroplast movement (Kasahara et al., 2002) as a module lacking in the Chl fluorescence decay (Cazzaniga et al., 2013). More recently it has been shown that qM has little influence on the photoprotection efficiency under high-light conditions in "shade"-grown Arabidopsis plants. Consequently, the existence of NPQ component qM and its influence on photoinhibition should be carefully reevaluated (Wilson and Ruban, 2020).

CONCLUSIONS

As drawn in this review, up-to-date research has shifted toward a dynamic role for NPQ and its components. From an alliance of this literature review, it is clear that NPQ response changes dynamically via its different components under light to balance between protection and light utilization. From our existing state of information, we suppose that the regulatory mechanisms of the different NPQ components play a dynamic role in the acclimation and adaptation of plants to fluctuating light environments as well as to other biotic stresses. It is well likely that the fundamental mechanisms of the NPQ components are the same for most of them. However, molecular players and regulatory elements that modulate these quenching types differ and might afford to adapt to the range of environmental conditions that photosynthetic organisms are experiencing. To end, I suggest that investigating the regulatory mechanisms of NPQ components and molecular players involved in these processes are promising targets for strategies to breed highly productive and tolerant crop plants.

REFERENCES

Amstutz C.L., Fristedt R., Schultink A., Merchant S.S., Niyogi K.K., Malnoë A. (2020) An atypical short-chain dehydrogenase reductase functions in the relaxation of photoprotective qH in Arabidopsis. *Nat. Plants*, **6:** 154–166.

Bellaflore S., Barneche F., Peltler G., Rochalx J.D. (2005) State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. *Nature*, **433:** 892–895; doi:10.1038/nature03286.

Betterle N., Ballottari M., Hienerwadel R., Dall'Osto L., Bassi R. (2010) Dynamics of zeaxanthin binding to the photosystem II monomeric antenna protein Lhcb6 (CP24) and modulation of its photoprotection properties. *Arch. Biochem. Biophys*., **504:** 67–77.

Blankenship R.E. (2002) Molecular mechanisms of photosynthesis. UK: Blackwell Science Ltd., Oxford, 336 p.

Briantais J.-M., Vernotte C., Picaud M., Krause G.H. (1979) A quantitative study of the slow decline of chlorophyll a fluorescence in isolated chloroplasts. *Biochim. Biophys. Acta, Bioenerg.*, **548:** 128-138 doi: 10.1016/0005-2728(79)90193-2.

Brooks M.D., Sylak-Glassman E.J., Fleming G.R., Niyogi K.K. (2013) A thioredoxin-like/-propeller protein maintains the efficiency of light harvesting in Arabidopsis. *Proc. Natl. Acad. Sci. USA*, **110:** E2733–E2740.

Cazzaniga S., Dall'Osto L., Kong S.G., Wada M., Bassi R. (2013) Interaction between avoidance of photon absorption, excess energy dissipation and zeaxanthin synthesis against photooxidative stress in Arabidopsis. *Plant J.*, **76:** 568–579; doi:10.1111/tpj.12314.

Clayton R.K. (1980) Photosynthesis: Physical mechanisms and chemical patterns. Cambridge: Cambridge University Press, 296 p.

Dall'Osto L., Caffarri S., Bassi R. (2005) A

mechanism of nonphotochemical energy dissipation, independent from PsbS, revealed by a conformational change in the antenna protein CP26. *Plant. Cell*, **17:** 1217-1232.

Dall'Osto L., Cazzaniga S., Wada M., Bassi R. (2014) On the origin of a slowly reversible fluorescence decay component in the Arabidopsis npq4 mutant. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **369:** 2013022.

Demmig-Adams B., Garab G., Adams W.W., Govindjee. (2014) Non-photochemical quenching and energy dissipation in plants, algae and cyanobacteria. The Netherlands, Dordrecht: Springer, 649 p.

Demmig-Adams B., Adams W.W. (1992) Photoprotection and other responses of plants to high light stress. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.*, **43:** 599–626.

Derks A., Schaven K., Bruce D. (2015) Diverse mechanisms for photoprotection in photosynthesis. Dynamic regulation of photosystem II excitation in response to rapid environmental change. *Biochim. Biophys. Acta*, **1847:** 468-485. 10.1016/j.bbabio.2015.02.008.

Goss R., Lepetit B. (2015) Biodiversity of NPQ. *J. Plant Physiol.*, **172:** 13–32 doi: 10.1016/j.jplph.2014.03.004.

Jahns P., Holzwarth A.R. (2012) The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. *Biochim. Biophys. Acta*, **1817:** 182–193.

Jeong M.S., Hwang E.Y., Jin G.-E., Park S.Y., Zulfugarov I.S., Moon Y.-H., Lee C.-H., Jang S.B. (2010) Expression and pH-dependence of the Photosystem II Subunit S from Arabidopsis thaliana. *Bull. Korean Chem. Soc.*, **31:** 1479-1484.

Johnson M.P., Ruban A.V. (2010) Arabidopsis plants lacking PsbS protein possess photoprotective energy dissipation. *The Plant Journal*, **61(2):** 283-289.

Johnson M.P., Ruban A.V. (2011) Restoration of rapidly reversible photoprotective energy dissipation in the absence of PsbS protein by enhanced ΔpH. *Journal of Biological Chemistry*, **286(22):** 19973-19981.

Johnson M.P., Zia A., Ruban A.V. (2012) Elevated ΔpH restores rapidly reversible photoprotective energy dissipation in Arabidopsis chloroplasts deficient in lutein and xanthophyll cycle activity. *Planta*, **235(1):** 193-204.

Kasahara M., Kagawa T., Olkawa K., Suetsugu N., Miyao M., Wada M. (2002) Chloroplast avoidance movement reduces photodamage in plants. *Nature*, **420:** 829–832, doi:10.1038/nature01213.

Krause G.H. (1988) Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physiol. Plant.*, **74:** 566-574.

Krause G.H., Weis E. (1991) Chlorophyll fluorescence and photosynthesis: the basics. *Annual Review of Plant Biology*, **42(1):** 313-349.

Krause G.H., Weis E. (1991) Chlorophyll fluorescence and photosynthesis: The basics. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.*, **42:** 313–349.

Krishnan-Schmieden M., Konold P.E., Kennis J., Pandit A. (2021) The molecular pH-response mechanism of the plant light-stress sensor PsbS. Nature communications, 12(1), 1-11.

Kromdijk J., Glowacka K., Leonelli L., Gabilly S.T., Iwai M., Niyogi K.K., Long S.P. (2016) Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science*, **354:** 857-861.

Li X.-P., Gilmore A.M., Caffarri S., Bassi R., Golan T., Kramer D., Niyogi K.K. (2004) Regulation of photosynthetic light harvesting involves intrathylakoid lumen pH sensing by the PsbS protein. *Journal of Biological Chemistry*, **279(22):** 22866-22874.

Liguori N., Campos S.R., Baptista A.M., Croce R. (2019) Molecular anatomy of plant photoprotective switches: the sensitivity of PsbS to the environment, residue by residue. *The Journal of Physical Chemistry Letters*, **10(8):** 1737-1742.

Malnoë A. (2018) Photoinhibition or photoprotection of photosynthesis, which one is it? Update on newly termed sustained quenching component, qH. *Environ. Exp. Bot.*, **154:** 123–133.

Malnoë A., Schultink A., Shahrasbi S., Rumeau D., Havaux M., Niyogi K.K. (2017) The plastid lipocalin LCNP is required for sustained photoprotective energy dissipation in Arabidopsis. *Plant. Cell*, **30:** 196–208.

Murchie E.H., Niyogi K.K. (2011) Manipulation of photoprotection to improve plant photosynthesis. *Plant Physiol.*, **155:** 86–92, doi:10.1104/pp.110.168831.

Müller P., Li X.-P., Niyogi K.K. (2001) Non-photochemical quenching. A response to excess light energy. *Plant Physiol.*, **125:** 1558–1566.

Nilkens M., Kress E., Lambrev P., Miloslavina Y., Müller M., Holzwarth A.R., Jahns P. (2010) Identification of a slowly inducible zeaxanthin-dependent component of non-photochemical quenching of chlorophyll fluorescence generated under steady-state conditions in Arabidopsis. *Biochim. Biophys. Acta*, **1797:** 466–475.

Niyogi K.K., Truong T.B. (2013) Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. *Curr. Opin. Plant Biol.*, **16:** 307-314 doi: 10.1016/j.pbi.2013.03.011.

Pashayeva A., Wu G., Huseynova I., Lee C.H., Zulfugarov I.S. (2021) Role of thylakoid protein phosphorylation in energy-dependent quenching of chlorophyll fluorescence in rice Plants. *International Journal of Molecular Sciences*, **22(15):** 7978.

Rees D., Noctor G., Ruban A.V., Crofts J., Young A., Horton P. (1992) pH dependent chlorophyll fluorescence quenching in spinach thylakoids from light treated or dark adapted leaves. *Photosynthesis Research*, **31(1):** 11-19.

Ruban A.V. (2012) The photosynthetic membrane. UK Chichester: John Wiley & Sons, Ltd., doi: 10.1002/9781118447628

Ruban A.V. (2016) Non-photochemical chlorophyll fluorescence quenching: Mechanism and effectiveness in protection against photodamage. *Plant Physiol.*, **170:** 1903–1916.

Ruban A.V., Johnson M.P., Duffy C.D.P. (2012) Photoprotective molecular switch in photosystem II. *Biochim. Biophys. Acta*, **1817:** 167–18; doi: 10.1016/j.bbabio.2011.04.007.

Ruban A.V., Horton P. (1995) An investigation of the sustained component of nonphotochemical quenching of chlorophyll fluorescence in isolated chloroplasts and leaves of spinach. *Plant. Physiol.*, **108:** 721–726.

Ruban A.V., Johnson M.P. (2009) Dynamics of higher plant photosystem cross-section associated with state transitions. *Photosynth. Res.*, **99:** 173–183.

Saccon F., Giovagnetti V., Shukla M.K., Ruban A.V. (2020) Rapid regulation of photosynthetic light harvesting in the absence of minor antenna and reaction centre complexes. *J. Exp. Bot.*, **71(12):** 3626-3637.

Sello S., Meneghesso A., Alboresi A., Baldan B., Morosinotto T. (2019) Plant biodiversity and regulation of photosynthesis in the natural environment. *Planta,* **249(4):** 1217-1228.

Walters R.G., Horton P. (1991) Resolution of components of non-photochemical chlorophyll fluorescence quenching in barley leaves. *Photosynth. Res.*, **27:** 121-133.

Wilson S., Ruban A.V. (2020) Rethinking the influence of chloroplast movements on non-photochemical quenching and photoprotection. *Plant. Physiol.*, **183:** 1213–1223.

Wraight C.A., Crofts A.R. (1970) Energy-dependent quenching of chlorophyll a fluorescence in isolated chloroplasts. *Eur. J. Biochem.*, **17:** 319–327; doi: 10.1111/j.1432-1033.1970.tb01169.x.

Zulfugarov I.S., Ham O.K., Mishra S.R., Kim J.Y., Nath K., Koo H. Y., Kim H.-S., Moon Y.-H., An G., Lee, C.-H. (2007) Dependence of reaction center-type energy-dependent quenching on photosystem II antenna size. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, **1767(6):** 773-780.

Zulfugarov I.S., Tovuu A., Dogsom B., Lee C.Y., Lee C.-H. (2010) PsbS-specific zeaxanthin-independent changes in fluorescence emission spectrum as a signature of energy-dependent non-photochemical quenching in higher plants. *Photochem. Photobiol. Sci.*, **9:** 697–703, doi: 10.1039/b9pp00132h.

Zulfugarov I.S., Tovuu A., Eu Y.J., Dogsom B., Poudyal R.S., Nath K., Hall M., Banerjee M., Yoon U.C., Moon Y.H., An G., Jansson S., Lee C.-H. (2014) Production of superoxide from Photosystem II in a rice (*Oryza sativa* L.) mutant lacking PsbS. *BMC Plant Biology*, **14(1):** 1-15.

Zulfugarov I.S., Wu G., Tovuu A., Lee C.-H. (2019) Effect of oxygen on the non-photochemical quenching of vascular plants and potential oxygen deficiency in the stroma of PsbS-knockout rice. *Plant Science*, **286:** 1-6.

Xlorofil flüoressensiyasının qeyri-fotokimyəsi sönməsi və onun komponentləri: son irəliləyişlər

Zülfüqarov İsmayıl Söhbət oğlu

AMEA-nın Molekulyar Biologiya və Biotexnologiyalar İnstitutu,
Bakı, Azərbaycan

Dəyişən işıq mühitinin təsirindən qorunmaq üçün bitkilər qoruyucu mexanizm kimi qeyri-fotokimyəvi sönmə (QFS) inkişaf etdirmişlər. QFS, fotosintetik orqanizmlərin fotosistem II-də singlet həyəcanlanmış xlorofilin (Xl) sönməsi yolu ilə artıq işıq enerjisinin istilik enerjisi kimi yayılmasından ibarətdir. Bu icmalda QFS və onun komponentləri haqqında bilinənləri ümumiləşdirdik. QFS komponentləri ilk növbədə həm QFS relaksasiyasına, həm də onun kimyəvi inhibitorlara qarşı həssaslığına görə fərqləndirilirdi. Bununla belə, çoxsaylı müxtəlif proseslər QFS-da iştirak edirlər, buna görə də QFS komponentlərinin formalaşmasında iştirak edən molekulyar iştirakçılara, eləcə də Xl flüoressensiyasının relaksasiya kinetikasına əsaslanaraq ayrılması təklif edilmişdir. Bu QFS növlərinə - enerjidən asılı sönmə (qE), vəziyyətə keçid sönə (qT), fotoinhibirləşdirici sönmə (qI), davamlı sönmə (qH), zeaksantindən asılı sönmə (qZ) və xloroplastın hərəkətindən asılı sönmə (qM) daxildir. Bu sönmə növlərini modullaşdıran molekulyar iştirakçılar və tənzimləyici elementlər bu icmalda müzakirə edilməsə də, onlar fərqli ola və bitkilərin məruz qaldığı ətraf mühit streslərə uyğunlaşmasına kömək edə bilərlər. Nəhayət, yüksək məhsuldar və dözümlü bitkilərinin yetişdirilməsi strategiyaları üçün perspektiv hədəflər kimi QFS komponentlərinin, onların molekulyar iştirakçılarının və QFS-i tənzim edən mexanizmlərin tədqiqinin rolu təklif edilmişdir.

Açar sözlər: *Xlorofil flüoressensiyası, xloroplastın hərəkətindən asılı sönmə, enerjidən asılı sönmə, qeyri-fotokimyəvi sönmə, bitki, vəziyyətə keçid sönmə, fotoinhibirləşdirici sönmə, davamlı sönmə, zeaksantindən asılı sönrmə*

Нефотохимическое тушение флуоресценции хлорофилла и его компоненты – последние достижения

Зульфугаров Исмаил Сохбат оглу

Институт молекулярной биологии и биотехнологий НАН Азербайджана, Баку, Азербайджан

В качестве защитного механизма в ответ на воздействие изменяющейся световой энергии растения развили нефотохимическое тушение (НФТ). НФТ включает тушение синглетно-возбужденного хлорофилла (*Xl*) в фотосинтезирующих организмах в фотосистеме II в виде тепловой энергии. В этом обзоре мы обобщили все, что известно о НФТ и его компонентах. Компоненты НФТ отличались в первую очередь релаксацией и чувствительностью к химическим ингибиторам. Однако в НФТ вовлечено множество различных процессов, поэтому было предложено провести разделение на основе отличий молекулярных составляющих, участвующих в формировании компонентов НФТ, а также флуоресценции *Xl*, на основе кинетики релаксации. Эти типы НФТ включают энергозависимое тушение (qE), тушение «переходов состояний» (qT), фотоингибирующее тушение (qI), непрерывное тушение (qH), зеаксантин-зависимое тушение (qZ) и тушение, зависящее от движения хлоропластов

(qM). Хотя молекулярные участники и регуляторные элементы, которые модулируют эти виды, в данном обзоре не обсуждаются, они могут быть разными и помогают растениям адаптироваться к стрессам окружающей среды, которым они подвергаются. В заключение, было высказано мнение о роли изучения компонентов НФТ, их молекулярных участников и механизмов, регулирующих НФТ, как перспективных мишеней для стратегий выращивания высокоурожайных и толерантных растений.

Ключевые слова: *Флуоресценция хлорофилла, тушение, зависящее от движения хлоропластов, энергозависимое тушение, нефотохимическое тушение, растение, тушение состояний, тушение фотоингибирования, непрерывное тушение, зеаксантин-зависимое тушение*

Journal of Life Sciences & Biomedicine, vol. 4(77), No 1, p. 84-89 (2022)

The seasonal dynamics of helminth invasion of domestic carnivores in Azerbaijan

Ibrahimova Reyhan Shohlat

Institute of Zoology, Azerbaijan National Academy of Sciences, Abbszade Str., Passage 1128, Block 504, Baku AZ1073, Azerbaijan
For correspondence: *ibrahimova_reyhan@bk.ru*

Received: March 31, 2022; Received in revised form: May 25, 2022; Accepted: June 08, 2022

The article is devoted to studying the seasonal variability in the degree of infection of domestic carnivores with helminths. The following results were obtained during the study: animals are infected by helminths to a greater extent in spring, summer, and autumn and to a considerably lesser extent in winter. Infection by trematodes is characterized by a 2-peak curve with peaks in spring and autumn. Cestodes infected domestic carnivores in spring, summer, and autumn with one peak in autumn, while nematodes were observed in animals in all seasons of the year. Investigation of seasonal dynamics of the degree of helminthic infection of domestic carnivores will allow making predictions about the extent, season, and the most dangerous period of infection of animals by helminths.

Keywords: *domestic carnivores (stray dogs, domestic cats), trematodes, cestodes, nematodes, season*

INTRODUCTION

Some authors studied the species composition and intermediate hosts of helminths of domestic carnivores (stray dogs, domestic cats) in different regions of Azerbaijan as well as some ecological factors that affect the degree of infection of these animals by helminths (Sadigov et al., 2003; Sadigov et al., 2001).

However, almost no scientific work has been done on studying the seasonal dynamics of infection of domestic carnivores by helminths in the Republic. The only paper concerning this problem is by M.Yolchiyev and R.Ibrahimova (2012) who studied the seasonal variation of helminthic infection in wild and domestic carnivores in the Shirvan region.

Study the seasonal dynamics of animal infection with helminths enables to specify some biological and ecological peculiarities of helminths on the one hand and on the other hand to identify the most dangerous periods i.e. the time of the highest degree of infection of animals by helminths. Thus, the study the effect of seasonal changes in the rate of infection of animals by helminths is practically important.

Taking into account the above mentioned, I conducted the investigation of seasonal dynamics of infection of domestic carnivores by helminths in Azerbaijan.

MATERIALS AND METHODS

The investigation was conducted in the territory of Azerbaijan in 2001- 2014 years. 349 stray dogs (105 in spring, 112 in summer, 73 in autumn, 59 in winter) and 323 domestic cats (105 in spring, 98 in summer, 63 in autumn and 57 in winter) were captured and treated through the widely accepted helminthological methods of body examination to study the effect of seasonal changes on infection of carnivores by common groups of helminths in various habitats: in the periaquatic areas, in the rural areas, in the urban areas and in the vicinity of the slaughterhouse. The animals were dead (they died due to unknown reasons or got hit by a car) (Skryabin, 1928).

The collected trematodes and cestodes were preserved in 70% ethanol, whereas nematodes were fixed in a 4% formalin solution. To identify the species belonging of trematodes and cestodes alum car-

10.5281/zenodo.7239809
Available online 30 June 2022

mine stained slides were prepared. Nematode species were treated in a solution of lactic acid and glycerol in a ratio of 1:1.

For the identification of trichinella, the various muscles of animals (diaphragm, ribs, thigh muscles) have been studied by the compressor method (Visotskaya, Daniel, 1973). The samples of muscles carded and made thinner, after adding lactic acid they tightened between slides. After 1 day until it becomes transparent, it was observed by trichinelloscope. The trichinella larvae are clearly visible under the microscope.

All helminth species were identified using MBS-6 and Olympus (Olympus SZ2=ST, *Olympus corporation*, Japan) stereomicroscopes.

The discovered helminths were identified according to DP Kozlov's (1977) reference book.

The prevalence was calculated with the following formula:

$$\boldsymbol{P} = \frac{\mathbf{X\ inf.}}{\mathbf{X\ anim.}} \boldsymbol{x}\ \mathbf{100}\%$$

RESULTS AND DISCUSSION

As the result of the investigation it was found that the rate of infection of animals by helminths varied among the different seasons. The percentage of infected animals was 30.3% in spring, 37.1% in summer, 20.7% in autumn and 16.4% in winter Table.

As a result of research, were found 36 species of helminths in domestic carnivores. Of these, 32 species in stray dogs, and 23 species in domestic cats.

It is seen from the Table that the studied animals were infected by 17 species of helminths in winter, 35 species in autumn, 34 species in spring and 24 species in summer. This fact is apparently related to the difference in population densities of intermediate hosts of helminths of domestic carnivores, which are most numerous in spring and autumn.

Fourteen species of 14 helminths (*Dipylidium caninum*, *Taenia hydatigena*, *T. pisiformis*, *Hydatigera taeniaeformis*, *Mesocestoides lineatus*, *Thominx aerophilus*, *Uncinaria stenocephala*, *Ancylostoma caninum*, *Toxoascaris leonina*, *T. canis*, *T. mystax*, *Molineus patens*, *Spirura rytipleurites*, *Physaloptera praeputiale*) were recorded in all seasons of the year.

The revealed helminths were present to a higher extent in spring, summer and autumn and to a considerably lesser extent in winter. Infection of domestic carnivores by trematodes was observed in spring and summer, by cestodes in spring, summer and autumn, and by nematodes throughout the whole year (Yolchuyev, Ibrahimova, 2012).

The infection of domestic carnivores by some trematode species Alaria *alata*, *Plagiorchis elegans*, *Cryptocotyle lingua,* which have freshwater snails as intermediate hosts, was observed only in spring and autumn.

The dynamics of infection of domestic carnivores by trematodes according to seasons is characterized by a two-peak curve. The first peak was observed in spring (May - 13.3%). At the beginning of summer (June), the level of infection considerably decreased (7.5%). The second peak was recorded in autumn (21.3%), while the lowest level of infection was observed in December - 3.5%.

The infection caused by a widespread trematode of the domestic carnivores, *A. alata* was highest in autumn -13.5%, spring-6.6%, and summer-7.5%. The highest infection of *P.cordatum* was observed in spring-15.3% and in autumn-4.5%; The highest incidence of *P.elegans* species was observed in autumn-9.1%.

The observed picture is due to the differences in the abundance of freshwater snails which are intermediate hosts of parasites and which populations exhibit considerably reduced activity in summer and winter because of high and low ambient temperatures respectively Fig.

The degree of infection of domestic carnivores with cestodes was characterized by a single-peak curve. Infection reached a peak in summer starting from 20.8% in spring, then sharply raised to 40.1% and decreased at the beginning of autumn in September, and the lowest level of infection 15.2% was registered in winter – December and January. Rodents, reptiles, cat and dog fleas, which are the intermediate hosts of cestodes are more active in the spring, summer and autumn months of the year, which causes infection of domestic carnivores by helminths in these seasons of the year (Ibragimova, 2017; Rzayev, 2006).

Table. Helminth species and their parameters of invasion of domestic carnivores in separate seasons

Families Species of helminths	Season of the year				Domestic carnivores	
	Spring	**Summer**	**Autumn**	**Winter**	**Stray dog**	**Domestic ca**
Trematoda						
Alaria alata Goeze, 1782	$\frac{6,6}{2-4}$	$\frac{7,5}{1-3}$	$\frac{13,5}{4-8}$	$\frac{3,5}{1-3}$	+	+
Plagiorchis elegans Rudolphi,1802	$\frac{3,3}{6}$	-	$\frac{9,1}{5-9}$	-	+	-
Echinochasmus perfoliatus Ratz,1908	$\frac{3,3}{8}$	-	$\frac{4,5}{11}$	-	+	-
Cryptocotyle lingua Creplin,1825	$\frac{2,9}{9}$	-	$\frac{3,1}{2-3}$	-	+	-
Pharhyngostomum cordatum Diesing,1850	$\frac{13,3}{2-17}$	-	$\frac{4,5}{15}$	-	+	+
C**estoda**						
Spirometra erinacei-europei Rudolphi,1819	$\frac{19,2}{1-11}$	-	$\frac{1,2}{1-7}$	$\frac{1,1}{2-6}$	+	+
Dipylidium caninum Lühe, 1758	$\frac{31,2}{2-16}$	$\frac{37,9}{5-32}$	$\frac{48,8}{1-20}$	$\frac{15,9}{3-12}$	+	+
Diplopylidium nolleri Skrjabin, 1924	$\frac{6,6}{3-7}$	$\frac{11,6}{1-6}$	$\frac{3,6}{4-9}$	$\frac{3,1}{1-4}$	+	+
D. skrjabini Popov, 1935	-	-	$\frac{9,1}{3-5}$	-	-	+
J.rossicum Skrjabin, 1923	$\frac{23,8}{3-14}$	$\frac{11,4}{1-12}$	$\frac{25,1}{5-16}$	-	-	+
Taenia hydatigena Pallas, 1766	$\frac{16,6}{2-14}$	$\frac{8,5}{1-7}$	$\frac{24,7}{1-14}$	$\frac{10,4}{3-6}$	+	+
T. crassiceps Zeder, 1880	$\frac{13,2}{2-7}$	$\frac{17,2}{3-14}$	$\frac{9,3}{2-5}$	-	-	+
T.laticollis Rudolphi, 1819	$\frac{6,5}{2-9}$	$\frac{7,9}{3-7}$	$\frac{4,5}{2-5}$	-	-	+
T.pisiformis Bloch, 1780	$\frac{15,9}{1-9}$	$\frac{22,5}{3-15}$	$\frac{18,1}{2-13}$	$\frac{15,9}{1-10}$	+	+
Hydatigera taeniaeformis Batsch,1786	$\frac{30,2}{1-10}$	$\frac{41,2}{1-21}$	$\frac{49,9}{3-27}$	$\frac{14,2}{2-6}$	+	-
Mesocestoides lineatus Goeze, 1782	$\frac{16,5}{2-20}$	$\frac{35,9}{8-27}$	$\frac{41,2}{5-12}$	$\frac{14,7}{6-14}$	+	+
Nematoda						
Capillaria plica Rudolphi, 1819	$\frac{10}{4-8}$	$\frac{6,8}{2-7}$	$\frac{9,1}{5-10}$	-	+	+
Thominx aerophilus Creplin, 1839	$\frac{5,8}{1-4}$	$\frac{12,5}{1-9}$	$\frac{4,6}{1-3}$	$\frac{3,9}{2-4}$	+	+
Trichocephalus georgicus Rodonaya,1950	$\frac{19,7}{1-8}$	-	$\frac{13,5}{1-6}$	-	+	-
Tr. vulpis Froelich, 1789	$\frac{23,8}{6-18}$	$\frac{9,7}{4-6}$	$\frac{13,5}{8-13}$	-	+	-
Trichinella spiralis Owen, 1825	-	-	$\frac{4,5}{23}$	$\frac{9,7}{14-29}$	+	-
Strongyloides vulpis Petrow, 1941	$\frac{18,7}{3-9}$	-	$\frac{14,8}{2-6}$	-	+	-
Ancylostoma caninum Ercolani, 1859	$\frac{23,3}{8-19}$	$\frac{36,1}{17-35}$	$\frac{47,8}{10-29}$	$\frac{16,8}{8-19}$	+	+
Uncinaria stenocephala Railliet, 1854	$\frac{28,5}{12-23}$	$\frac{37,2}{17-42}$	$\frac{49,6}{9-32}$	$\frac{17,4}{10-21}$	+	+
Molineus patens Dujardin, 1945	$\frac{17,6}{3-9}$	$\frac{19,7}{2-8}$	$\frac{12,1}{1-6}$	$\frac{9,7}{1-5}$	+	+
Toxascaris leonina Linstow, 1902	$\frac{24,8}{3-6}$	$\frac{39,1}{2-14}$	$\frac{50,3}{1-14}$	$\frac{18,3}{2-8}$	+	+
Toxocara canis Werner, 1782	$\frac{23,8}{1-14}$	$\frac{17,2}{3-15}$	$\frac{22,8}{2-11}$	$\frac{15,4}{2-7}$	+	-
T. mystax Zeder, 1800	$\frac{14,9}{3-16}$	$\frac{17,4}{2-11}$	$\frac{31,7}{1-14}$	$\frac{11,4}{1-7}$	+	+
Spirura rytipleurites Deslongchamps,1824	$\frac{9,1}{4-9}$	$\frac{5,2}{3-7}$	$\frac{4,8}{1-6}$	$\frac{2,9}{2-4}$	+	+
Spirocerca arctica Petrow,1927	$\frac{18,7}{2-8}$	$\frac{19,8}{2-9}$	$\frac{14,6}{2-5}$	-	+	-
Sp.lupi Rudolphi, 1809	$\frac{9,1}{3-5}$	$\frac{9,6}{2-5}$	$\frac{4,5}{7}$	-	+	-
Physaloptera praeputiale Linstow,1888	$\frac{18,9}{2-7}$	$\frac{21,7}{2-9}$	$\frac{12,4}{1-5}$	$\frac{9,7}{2-5}$	+	+
Ph. sibirica Petrow et Gorbunow,1931	$\frac{10,9}{8-17}$	$\frac{9,1}{5-12}$	-	-	+	-
Rictularia affinis Jagerskiold,1904	$\frac{15,7}{6-19}$	-	$\frac{9,1}{10-15}$	-	+	-
R.cahirensis Jagerskiold, 1904	$\frac{11,4}{3-5}$	-	$\frac{13,5}{5-9}$	-	+	+
Dirofilaria repens Railliet et Henry,1911	$\frac{7,9}{1-7}$	$\frac{17,2}{1-12}$	$\frac{18,1}{5-12}$	-	+	+
Total: 36	**34**	**24**	**35**	**17**	**32**	**23**

Remark: *above figures refers to percentage of infection, the below figures refers to prevalence (from min to max)*

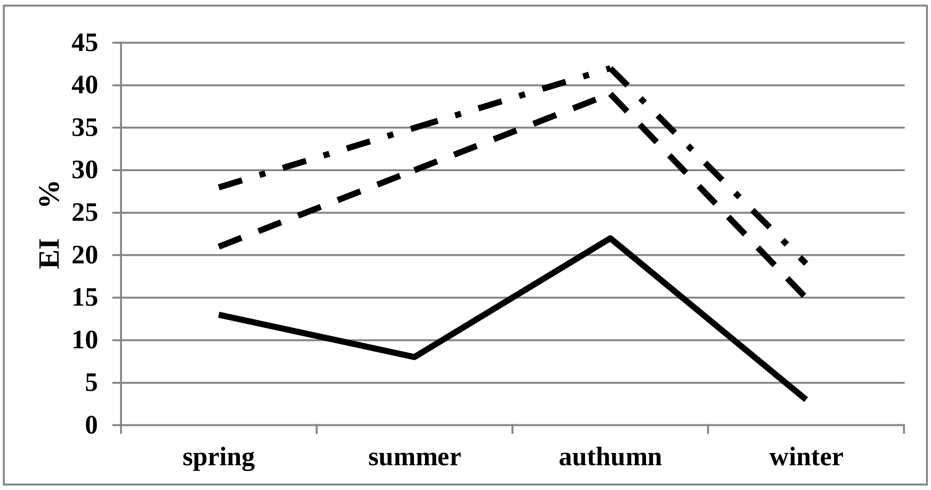


Fig. Seasonal dynamics of the degree of infection of domestic carnivores by helminths in Azerbaijan.

The following species of helminths infected domestic carnivores mainly in spring, summer and autumn. These include *D.caninum*, *Physaloptera sibirica*, *Rictularia cahirensis*, *Dirofilaria repens*, which intermediate hosts are various species of invertebrates; *Joyeuxiella rossicum*, *Mesocestoides lineatus*, *H. taeniaeformis*, *H. krepkogorski*, *Trichinella spiralis* which intermediate hosts are rodents; and *Spirometra erinacei-europei*, *Diplopylidium nolleri*, *D. skrjabini* which intermediate hosts are small reptiles.

The degree of infection of domestic carnivores by *D. caninum* constituted 31.2% in spring, 37.9% in summer, and 48.8% in autumn; by *M. lineatus* 16.5% in spring, 35.9% in summer and 41.2% in autumn; by *H. taeniaeformis* 30.2% in spring, 41.2% in summer and 49.9% in autumn.

The reason as we have mentioned is the favorable climatic conditions for the growth of helminths and their eggs in spring, summer, and autumn, and the animals the constant contact with rodents, reptiles, insects and snails, which are intermediate hosts of these trematodes. During winter time, because of dry and cold weather conditions some helminths' eggs are destroyed in the soil and the weakening or interruption of the contact between animals and intermediate hosts (rodents, reptiles, and insects) are very poor, and sometimes never happened.

It has been recorded that domestic carnivores are infected with nematodes in all seasons of the year in high intensity.

Infection of animals with nematodes is characterized by the 1-peak curve. Infection of animals started from 30.2% in spring months and reached its peak of 43.2 in summer, then again dropped to 18.7% in winter due to the absence of optimal temperatures needed for the development of helminths and their eggs.

As nematodes are geohelminths and their development and distribution depend on environmental abiotic factors. Eggs of geohelminths survive for about 17 months in soil (Guliyeva, 1989). When the temperature and humidity are favorable, the egg could switch to an invasive state and cause infection to animals in any season of the year.

In the studied areas, the most frequently found geohelminths were *A. caninum* - `16.8% in winter, 23.3% in spring, 36.1% in summer, 47.8% in autumn, *U. stenocephala* - 17.4% in winter, 28.5% in spring, 37.2% in summer, 49.6% in autumn, *T. leonina* - 18.3% in winter, 24.8% in spring, 39.1% in summer, 50.3% in autumn.

In various animal nurseries in Russia, a considerable difference in the degree of helminth infection of dogs between the spring season (April – 64.5%) and winter season (20.7%) was observed (Malykhin, Vasilevich, 2004; Trunova, Nurmagomedova, 2017; Zakharov, 2000).

As a result of the research, it has been found that there is a considerable difference in the baseline of seasonal spreading and growth of helminths. Because of the impact of abiotic factors in the area, it is necessary to give predictions on helminth infections for each area individually and at different times, and the control measures should be based on it.

So, by studying the seasonal dynamics of dominant helminths of carnivores in the territory of Azerbaijan, it is possible to make predictions about the extent, season, and dangerous period of infection of animals with helminths and to take preventive measures.

REFERENCES

Guliyeva R. (1989) Epidemiological basics of prevention and larval toxocariasis (on the example of Baku city agglomeration). *Authoreferate of cand. diss*. Baku: 21 pp.

Ibragimova R. (2017) The role of intermediate hosts in the distribution of helminths of domestic carnivorous animals in Azerbaijan. *Journal of the Belarusian State University. Biology,* **No3:** 94-100.

Kozlov D. (1977) Determinant of helminths carnivores of USSR. M.: Nauka, 275 p.

Malykhin Ye., Vasilevich F. (2004) Helminthofauna of dogs in Moscow city. *Materials of International scientific-practical conference of young scientists "Contribution of young scientists to the agrarian Science in XXI century*. Ryazan: p. 429-431.

Rzayev N. (2006) Study of biology of Mollusks *Gyraulus albus* (Miller, 1774), intermediate hosts of paramfistomaty in Lankaran Natural Area. *Tr. Institute of Zoology of Azerbaijan NAS,* **XXVIII:** 766-780.

Sadıgov I., Yolchuyev M., Ibrahimova R. (2001) Ecological properties of main helminthiasis agents of carnivorous animals in Absheron and adjacent south-eastern regions. "*The study and protection of fauna at the end of XX century*", dedicated to 80th anniversary of *Academician M.A. Musayev,* Baku: p. 191-193.

Sadıgov I., Yolchuyev M., Ibrahimova R. (2003) The study of effect of residential areas on helminth fauna of homeless dogs in Azerbaijan. 1st *conference of Azerbaijan Zoologists Society,* Baku: p.126-129

Skryabin K. (1928) Methods of full helminthological examination of the vertebrates, including human. M.: Publishing house of Moscow State University, 45 p.

Trunova S., Nurmagomedova S. (2017) Seasonal dynamics of helminth infection of dogs in the plain zone of Dagestan. *Russian Parasitological Magazine.* **4 (42):** 358-360.

Visotskaya S., Daniel M. (1973) Parasitological research methods. L.: Nauka, 70 p.

Yolchuyev M., Ibrahimova R. (2012) Seasonal and age dynamics of helminths of wild and domestic carnivorous animals in Shirvan region. *Proceedings of ANAS Institute of Zoology,* **30(2):** 93-98

Zakharov P. (2000) Helminthiasis in guard dogs in the territory of megalopolis. *8-th International Congress on the problems of the veterinary medicine of small animals.* Moscow: p. 184 – 185.

Azərbaycanda əhli ətyeyən heyvanların helmintlərlə yoluxmasının fəsil dinamikasi

İbrahimova Reyhan Şöhlət qızı

AMEA-nın Zoologiya İnstitutu, Bakı, Azərbaycan

Məqalə əhli ətyeyən heyvanların fəsillər üzrə helmintlərlə yoluxma dinamikasına həsr edilmişdir. Tədqiqat zamanı müəyyən edilmişdir ki, trematodlarla yoluxması yalnız yaz və payız aylarında 2 zirvəli əyri ilə; sestodlarla - yaz, yay və payız aylarında 1 zirvəli; nematodlarla ilin bütün fəsillərində yoluxaraq 1 zirvəli əyri ilə xarakterizə olunur və yoluxmaları da uyğun olaraq yazda 32,3%, yayda 34,1%, payızda 18,7%, qışda isə 12,4% təşkil etmişdir. Əhli itkimi və pişikkimilərin geniş yayılmış helmintlərlə fəsillər üzrə yoluxmasını müəyyən etməklə, heyvanların helmintlərlə hansı fəsildə yüksək ekstensivliklə yoluxması və yoluxmanın təhlükəli dövrləri barədə əvvəlcədən proqnoz vermək və onlara qarşı qabaqlayıcı tədbirləri hazırlamaq olar.

Açar sözlər: *Əhli ətyeyən heyvanlar (sahibsiz itlər, ev pişikləri), trematodlar, sestodlar,nematodlar, fəsillər*

Сезонная динамика степени зараженности гельминтами домашних плотоядных животных в Азербайджане

Ибрагимова Рейхан Шохлат кызы

Институт зоологии НАН Азербайджана, Баку, Азербайджан

Статья посвящена изучению сезонной изменчивости степени зараженности гельминтами домашних плотоядных животных. В ходе исследования были получены следующие результаты: животные заражаются гельминтами в большей степени весной, летом и осенью и в значительно меньшей степени зимой. Заражение трематодами происходит только весной и осенью и характеризуется двупиковой кривой; при цестодах заражение наблюдается весной, летом и осенью – кривая при этом однопиковая; зараженность нематодами наблюдается в течение всех сезонов года, при этом форма кривой также однопиковая. В процентном выражении зараженность нематодами составляет весной 32,3%, летом 34,1%, осенью 18,7% и зимой 12,4%. Изучение сезонной динамики степени зараженности гельминтами домашних плотоядных животных позволит прогнозировать опасные периоды заражения и разработать профилактические меры борьбы против них.

Ключевые слова: *Домашние плотоядные (бродячие собаки, домашние кошки), трематоды, цестоды, нематоды, сезон*

Journal of Life Sciences & Biomedicine, vol. 4(77), No 1, p. 90-96 (2022)

Investigation of C609T polymorphism in the *NQO1* gene in patients diagnosed with colorectal cancer in the Azerbaijani population

[1]*Bayramov Bayram Ilham, [2] Bayramov Nuru Yusif

[1] *Genetic Resources Institute, Azerbaijan National Academy of Sciences, 155 Azadlig Ave., Baku AZ1106, Azerbaijan*
[2] *Azerbaijan Educational-Surgical Clinic, Azerbaijan Medical University, 23 A. Bakikhanov Str., Baku AZ1022, Azerbaijan*
***For correspondence:** bayram-bayramov-90@hotmail.com*

Received: May 02, 2022; Reviewed: May 30, 2021; Accepted: June 12, 2022

This study aimed to determine the clinical significance of the *NQO1* C609T (rs1800566, Pro187Ser) polymorphism in patients with colorectal cancer (CRC) in the Azerbaijani population. 142 patients with CRC and 146 healthy individuals were included in the study. DNA from blood was isolated using the salting-out method, and genotypes were determined on agarose gel using the PCR-RFLP method. When comparing the patients and control groups, heterozygous CT (OR=1.813; 95%CI=1.097–2.995, P=0.020), under the dominant model (OR=1.842; 95%CI=1.137–2.983, P=0.013), and the mutant T allele (OR=1.644; 95%CI=1.096–2.465, P=0.016) were statistically associated with an increased risk of CRC. When comparing male patients with healthy men, heterozygous CT was associated with a higher risk of CRC (OR=2.219; 95%CI=1.079-4.565, P=0.029). However, the age, pathological tumor grade and stage, smoking, and alcohol consumption of the study groups were compared and no significant relationship was found (P>0.05). Our findings showed that the *NQO1* C609T polymorphism is related to the risk of colorectal cancer in the Azerbaijan population.

Keywords: *Colorectal cancer, polymorphism, PCR-RFLP, NQO1, C609T*

INTRODUCTION

Colorectal cancer (CRC) is the most common cause of cancer death worldwide in both men and women (Siegel et al., 2018). Early detection and excision of precancerous lesions with screening programs have been associated with a reduced risk of CRC in different populations (Holme et al., 2013; Welch and Robertson, 2016). Genetic profiling of tumors and detection of somatic genetic variants are necessary and provide opportunities for effective treatment choices (Biller and Schrag, 2021). Furthermore, the underlying genetic causes of CRC include Chromosome instability (CIN) and Microsatellite instability (MSI). CIN phenotype includes structural abnormalities in chromosomes (copy number alterations, duplication, amplification, deletion, insertion, loss of heterozygosity) and chromosomal number changes such as aneuploidy which is present in 65-70% of all CRC cases (Nguyen et al., 2020). Microsatellites are DNA sequences containing repetitive motifs that tend to accumulate high mutation rates. MSI inactivates DNA repair system genes, including *MLH1, MSH2, MSH3, MSH6,* and *PMS2* (Li, 2008).

NQO1 is a multifunctional antioxidant encoding gene and plays an essential role in protecting cells from oxidative stress. NQO1 acts as an anti-cancer enzyme. In some cancers (breast, colon, lung cancer, etc.), the expression of the *NQO1* gene is increased (Yadav et al., 2018). *NQO1* is a gene of approximately 20 kb in length, consisting of 6 exons on chromosome 16q22.1 (Iskander and Jaiswal, 2005). Several polymorphisms have been identified in the *NQO1* gene (Nebert et al., 2002). However, the most studied and clinically significant is polymorphism C609T (rs1800566, P187S), which converts proline amino acid to serine in exon 6 (Yadav et

10.5281/zenodo.7239850
Available online 30 June 2022

al., 2018). *NQO1* C609T polymorphism has been widely investigated among different ethnic groups for CRC susceptibility, but the results are inconsistent.

Several different clinical studies have been performed on the *NQO1* gene C609 polymorphism, indicating that the TT genotype has an increased risk of disease in patients with kidney, urothelial, esophageal, bladder, breast and stomach cancers, pediatric and adult leukemia compared to healthy controls (Sameer et al., 2010). There are no consistent data available to show the association between *NQO1* gene polymorphism and CRC risk in our population. In this case-control study, we investigated for the first time the association between *NQO1* C609T (rs1800566, Pro187Ser) gene polymorphisms and subjects' age, gender, clinicopathological parameters, smoking and alcohol use parameters, and CRC susceptibility in the Azerbaijani population.

MATERIALS AND METHODS

This study included 142 patients diagnosed with colorectal cancer at the Azerbaijan Medical University and the Scientific Surgery Center named after M. Topchubashov and 146 healthy people as a control group. The control group was selected from individuals with no history of inflammatory bowel disease or cancer. Cancer biopsy samples taken from patients were evaluated due to pathohistological analysis, and the stage and grade of the tumor were determined. Venous blood was taken from the study groups in tubes with EDTA. DNA extraction was carried out in the Laboratory of Human Genetics of ANAS Institute of Genetic Resources. DNA extraction from blood samples was performed according to the protocol by the *Salting-out* method. Quantitative and qualitative parameters of DNA were measured by the spectrometric method (Thermo Scientific™ NanoDrop™ 2000/2000c). Then the amplification process was performed by PCR. The sequence of specific primers used in PCR follows forward 5’-AAGCCCAGACCAACTTCT-3’, reverse 5’-TCTCCTCATCCTGTACCTCT-3. The PCR reaction with a total volume of 20 µl consisted of the following: 2µl DNA (200 ng/µl), 2 µl 10X buffer [10 mMTris–HCl pH 8.0, 50 mM KCl], 2 µl $MgCl_2$, dNTP mixture 0.2 µl (20 mM), 0.5 µl (100 µM) and 0.2 µl (5 U/µl) from each primer, 0.2 µl (5 U/µl) Taq DNA polymerase (Solis BioDyne, Tartu, Estonia) and 12.6 µl distilled water (dH_2O). The amplification condition for PCR (Applied Biosystems, USA) consisted of 5 minutes at 95ºC, 35 cycles of 30 seconds at 95ºC, 45 seconds at 59ºC and 2 minutes at 72ºC, then final elongation of 7 minutes at 72ºC. *HinfI* (NEB, New England Biolabs) restriction enzymes were used to identify *NQO1* C609T genotypes. Restriction fragments were visualized on 2% agarose gel stained with Ethidium Bromide under the UV gel documentation system. As a result of digestion of PCR products with the restriction enzyme, wild type CC 271 bp, heterozygous CT 271 bp, 151 bp and 120 bp, and homozygous mutant TT genotype 151 bp and 120 bp (Figure).

Statistical analysis. Statistical analysis using *SPSS* version 17.0 (SPSS Inc, Chicago, USA) Pearson's chi-squared test was used for genotype and allele frequency comparison. Odds ratios (OR) and confidence intervals (CI) were calculated to estimate relative risk. A *P* value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The study involved 142 patients with colon cancer and 146 practically healthy individual control groups. Demographic parameters for patients and controls are shown in Table 1. The patient group consisted of 84 men and 58 women, and the control group consisted of 64 men and 82 women, respectively. The age range of patients was 25-85, and in the control group, it was 27-82 years. No statistical association was observed when the study groups were compared by age (P=0.256). Furthermore, 8.2% of patients had G1, 91% G2 and 24.7% G3 tumors, and 2.1% T1, 11.3% T2 and 78.9% T3, and 7.7% had a tumor stage. In addition, no significant statistical difference was observed when comparing research groups in terms of data on smoking and alcohol consumption (P>0.05).

The genotype and allele frequencies of the C609T polymorphism of the *NQO1* gene are shown in Table 2. Genotypic frequencies were found in 54.9% CC, 39.5% CT and 5.6% TT in patients, and 69.2% CC, 27.4% CT and 3.4% TT in the control group, respectively. Heterozygous

CT and homozygous mutant TT were higher in patients. A statistical association was found between heterozygous CT and increased risk of colon cancer (OR=1.813; 95% CI=1.097–2.995; P=0.020). Although the association was found in the dominant model (OR=1.842; 95% CI=1.137–2.983; P=0.013), no association was found in the recessive model (P>0.05). In addition, 74.6% of patients had normal C allele, and 25.4% had mutant T allele. 82.9% had a wild type C allele in the control group, and 17.1% had a mutant T allele. A statistical association was observed between the mutant T allele (OR=1.644; 95% CI=1.096–2.465; P=0.016) and the risk of colorectal cancer.

Table 1. Clinical and demographic parameters of study groups

	Patients N=142 (%)	Controls N=146 (%)	*P* value
Gender			
Male	84 (59.2)	64 (43.8)	0.256
Female	58 (40.8)	82 (56.2)	
Age			
Age interval	25-85	27-82	
Mean	61±9.1	60±10.3	
Histological Grade			
G1	13 (8.2)		
G2	91 (67.1)		
G3	38 (24.7)		
Tumor Stage			
T1	3 (2.1)		
T2	16 (11.3)		
T3	112 (78.9)		
T4	11 (7.7)		
Smoking Status			
Smokers	49 (34.4)	51 (34.9)	0.942
Non-Smokers	85 (60)	89 (60.9)	
Unknown	8 (5.6)	6 (4.2)	
Alcohol consumption			
Yes	45 (31.7)	46 (31.5)	0.368
No	89 (62.7)	94 (64.4)	
Unknown	8 (5.6)	6 (4.1)	

When comparing genotypes by gender factor (Table 3), 53.6% of wild-type CC, 40.5% of heterozygous CT, and 5.9% of mutant TT were found in male patients. In healthy men, the genotypic frequencies were 73.4% CC, 25% CT and 1.6% TT, respectively. A statistically significant association was found between heterozygous CT genotype and increased risk of colorectal cancer among men included in the study (OR=2.219; 95%CI=1.079-4.565; P=0.029). Moreover, genotypic frequencies in female patients were 56.9% CC, 37.9% heterozygous CT, and 5.2% mutant TT, respectively. It was determined as 65.9% CC, 29.3% CT, and 4.8% TT in healthy women. Although both CT and TT genotypes were more common in female patients, no statistical difference was found between genotypes and disease risk (P<0.05).

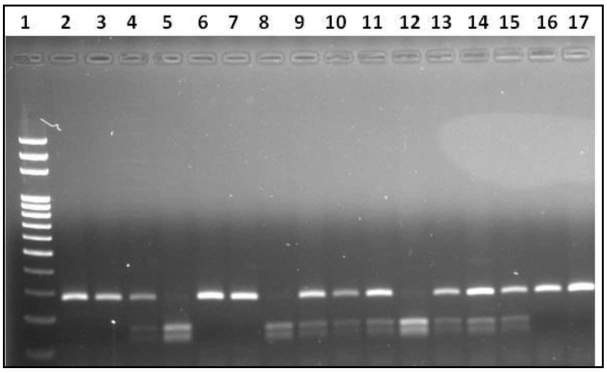


Fig. Genotypes of *NQO1* C609T polymorphism determined by PCR-RFLP methods in agarose gel
DNA Ladder (100 bp): Lane-1; **Wild type CC:** Lane-2, 3, 6, 7, 16, 17; **Heterozygous CT:** Lane-4, 5, 9, 10, 11, 13, 14, 15; **Homozygous mutant TT:** Lane-5, 8, 12.

The distribution of genotypes according to the average age in the study groups is presented in Table 4. The distribution of genotypes in patients under 60 years of age was determined by 57.7% CC, 36.5% CT and 5.8% TT, and in the control group, 68.9% CC, 28.3% CT and 2.8% TT, respectively. Although heterozygous CT (OR=1.541; 95%CI=0.754-3.150; P=0.234) and mutant TT (OR=2.433; 95%CI=0.465-4.514; P=0.364) genotypes were more common in patients compared with the control group. A statistical relationship between genotypes and disease risk was not found when comparing patients under 60 with the control group. However, in patients over 60 years of age, normal CC, heterozygous CT, and mutant TT genotypes were 53.3%, 41.1%, and 5.6%, while these frequencies were 70%, 25%, and 5% in the controls, respectively. Both CT (OR=2.158; 95%CI=0.932-4.998; P=0.069) and TT (OR=1.458; 95%CI=0.265-3.021; P=0.663) genotypes are more common in patients over 60 years of age. No statistically significant results were obtained between genotypes and disease risk when comparing study groups over 60 years of age (P>0.05).

Genotypic frequencies were found in non-smokers compared to non-smokers (Table 5), with 27.3% CC, 52.7% CT and 20% TT in smokers. In non-smokers, the distribution of genotypes was 38.9% CC, 42.2% CT and 18.9% TT, respectively. Although heterozygous CT (OR=0.952; 95%CI=0.460-1.972; P=0.895) and mutant TT (OR=0.667; 95%CI=0.121-3.678; P=0.642) are more common in smokers, this difference was not statistically significant. Additionally, comparing genotypes in terms of alcohol use, heterozygous CT (49%) and homozygous TT (20.4%) were more prevalent in alcohol users. However, no association was found between genotypes CT (OR=1.619; 95%CI=0.772-3.397; P=0.201) and TT (OR=0.971; 95%CI=0.175-5.407; P=0.974) and disease risk.

The distribution of genotype frequencies in tumor grade and the stage is presented in Table 6. Both heterozygous CT genotype (47.4%) and mutant TT genotype (7.9%) were prevalent in malignant tumor G3. The heterozygous CT genotype was more common in the T3 and T4 stages of the tumor, and the mutant TT genotype was more common in T3. No statistical correlation was found between tumor grade and stages and C609T polymorphism (P>0.05).

Table 2. Distribution of genotype and allele frequencies of C609T polymorphism of *NQO1* gene in subject groups

	Patients (N=142 (%))	**Controls (N=146 (%))**	**OR (95%CI)**	***P* value**
Genotypes				
CC	78 (54.9)	101 (69.2)	1	-
CT	56 (39.5)	40 (27.4)	1.813 (1.097-2.995)	**0.020**
TT	8 (5.6)	5 (3.4)	2.072 (0.652-6.581)	0.209
Dominant				
CC	78 (54.9)	101 (69.2)	1	-
CT+TT	64 (45.1)	45 (30.8)	1.842 (1.137-2.983)	**0.013**
Recessive				
CC+CT	134 (94.4)	141 (96.6)	1	-
TT	8 (5.6)	5 (3.4)	1.684 (0.537-5.275)	0.367
Allele				
C	212 (74.6)	242 (82.9)	1	-
T	72 (25.4)	50 (17.1)	1.644 (1.096-2.465)	**0.016**

Table 3. Distribution of genotype frequencies between men and women

Males	**Patients (N=84 (%))**	**Controls (N=64 (%))**	**OR (95%CI)**	***P* value**
Genotypes				
CC	45 (53.6)	47 (73.4)	1	-
CT	34 (40.5)	16 (25)	2.219 (1.079-4.565)	**0.029**
TT	5 (5.9)	1 (1.6)	2.315 (0.587-3.396)	0.205
Females	**N=58** (%)	**N=82** (%)		
Genotypes				
CC	33 (56.9)	54 (65.9)	1	-
CT	22 (37.9)	24 (29.3)	1.500 (0.728-3.090)	0.270
TT	3 (5.2)	4 (4.8)	1.227 (0.258-5.831)	0.798

Table 4. Distribution of genotype frequencies by age factor

Age, ≤ 60	**Patients (N=52 (%))**	**Controls (N=106 (%))**	**OR (95%CI)**	***P* value**
CC	30 (57.7)	73 (68.9)	1	-
CT	19 (36.5)	30 (28.3)	1.541 (0.754-3.150)	0.234
TT	3 (5.8)	3 (2.8)	2.433 (0.465-4.514)	0.364
> **60**	N=90 (%)	N=40 (%)		
CC	48 (53.3)	28 (70)	1	-
CT	37 (41.1)	10 (25)	2.158 (0.932-4.998)	0.069
TT	5 (5.6)	2 (5)	1.458 (0.265-3.021)	0.663

Table 5. Comparison of genotype frequencies in terms of smoking and alcohol use

Genotypes	Smokers (N=49 (%))	Non-smokers (N=85 (%))	OR (95%CI)	*P* value
CC	27 (27.3)	45 (38.9)	1	-
CT	20 (52.7)	35 (42.2)	0.952 (0.460-1.972)	0.895
TT	2 (20)	5 (18.9)	0.667 (0.121-3.678)	0.642
	Drinkers (N=45)	**Non-drinkers (N=89)**		
CC	21(30.6)	51 (36.5)	1	-
CT	22 (49)	33 (44.7)	1.619 (0.772-3.397)	0.201
TT	2 (20.4)	5 (18.8)	0.971 (0.175-5.407)	0.974

Table 6. Distribution of genotype frequencies in the degree and stage of the tumor

Tumor Grade	CC (N (%))	CT (N (%))	TT (N (%))	*P* value
G1	7 (53.8)	6 (46.2)	0	0.397
G2	54 (59.3)	32 (35.2)	5 (5.5)	
G3	17 (44.7)	18 (47.4)	3 (7.9)	
Tumor Stage				
T1	0	3	0	0.201
T2	10 (62.5)	6 (37.5)	0	
T3	61 (54.5)	43 (38.4)	8 (7.1)	
T4	4 (36.4)	7 (63.6)	0	

In summary, in our study, we investigated the C609T polymorphism of the *NQO1* gene in patients diagnosed with colorectal cancer and in the control group. We determined the relationship between polymorphism and disease risk and clinical parameters. Thus, there was a statistically significant association between heterozygous CT, dominant model (CC/CT+TT) and mutant T allele and colorectal cancer risk in our study. Similar findings supporting our findings were reported in a study presented by Wang and colleagues. Researchers have provided strong evidence for an association between the *NQO1* gene C609T polymorphism and the risk of colorectal cancer. They have shown that the mutant T allele is a significant risk factor for disease (Wang et al., 2013). Another study found that the T allele was significantly associated with susceptibility to colorectal cancer in both Asians and Caucasians. There was also a positive association between *NQO1* C609T polymorphism and susceptibility to disease in smokers compared to non-smokers (Zheng et al., 2014). In our study, no association was found between genotypes and disease risk compared to smokers and non-smokers. A meta-analysis of 12 studies that included 5,525 patients and 6,272 healthy individuals reported that the mutant T allele was a high-risk factor for colorectal cancer and received a positive statistical correlation for the dominant model (Ding et al., 2012). A meta-analysis of another Asian population showed a clear association between *NQO1* Pro187Ser polymorphism and the risk of colorectal cancer (Zhao et al., 2014). In a study by Yadav and colleagues, C609T polymorphism in the *NQO1* gene was found to increase the risk of colorectal cancer and other parts of the digestive tract, especially gastric cancer (Yadav et al., 2018). In addition, no association was observed in our study between *NQO1* gene polymorphism and disease risk in patients who smoked and consumed alcohol. A study by Peng and colleagues found that *NQO1* 609 C>T polymorphism increased the risk of colorectal cancer in the Chinese population, especially in patients who smoked and drank alcohol (Peng et al., 2013). A 2010 study of the Kashmir population also found an association between *NQO1* C609T polymorphism and the risk of developing colorectal cancer and a statistical relationship between smoking, age, cancer stage, and degree of homozygous mutant genotype (Sameer et al., 2010). In comparison with this study, our study did not find a statistically significant relationship between cancer stage and degree, age factor, smoking and alcohol use factors, and *NQO1* C609T polymorphism.

In summary, in our case-control study, heterozygous CT and mutant T alleles were associated with an increased risk of CRC in our population. In particular, a statistically significant association was found between the *NQO1* CT genotype and in-

creased CRC risk when male patients were compared to healthy men. Thus, our results emphasize that the *NQO1* gene C609T polymorphism may play an essential role in the molecular pathogenesis of colorectal cancer and suggest that it may be a genetic marker in early detection.

REFERENCES

Biller L.H., Schrag D. (2021) Diagnosis and treatment of metastatic colorectal cancer: A review. *JAMA*, **325(7):** 669-685.

Ding R., Lin S., Chen D. (2012).. Association of NQO1 rs1800566 polymorphism and the risk of colorectal cancer: a meta-analysis. *Int. J. Colorectal Dis*., **27(7):** 885-892.

Holme Ø., Bretthauer M., Fretheim A., Odgaard-Jensen J., Hoff G. (2013).. Flexible sigmoidoscopy versus faecal occult blood testing for colorectal cancer screening in asymptomatic individuals. *Cochrane Database Syst Rev*., **(9):** CD009259.

Iskander K., Jaiswal A.K. (2005) Quinone oxidoreductases in protection against myelogenous hyperplasia and benzene toxicity. *Chem Biol Interact*., **153-154:** 147-157.

Li G.M. (2008) Mechanisms and functions of DNA mismatch repair. *Cell Res*., **18:** 85-98; doi: 10.1038/cr.115

Nebert D.W., Roe A.L., Vandale S.E., Bingham E., Oakley G.G. (2002) NAD(P)H: quinoneoxidoreductase (NQO1) polymorphism, exposure to benzene, and predisposition to disease: a HuGE review. *Genet Med*., **4: 62-70.**

Nguyen L.H., Goel A., Chung D.C. (2020) Pathways of colorectal carcinogenesis. *Gastroenterology*, **158:** 291–302.

Peng X.E., Jiang Y.Y., Shi X.S., Hu Z.J. (2013) NQO1 609C>T polymorphism interaction with tobacco smoking and alcohol drinking increases colorectal cancer risk in a Chinese population. *Gene*, **521(1):** 105-110.

Sameer A.S., Shah Z.A., Syeed N., Rasool R., Afroze D., Siddiqi M.A. (2010) NAD(P)H:quinone oxidoreductase 1 (NQO1) Pro187Ser polymorphism and colorectal cancer predisposition in the ethnic Kashmiri population. *Asian Pac. J. Cancer Prev*., **11(1):** 209-213.

Siegel R.L., Miller K.D., Jemal A. (2018) Cancer statistics, CA. *Cancer J. Clin.,* **68:** 7–30.

Wang Y., Zhang G., Luo Y. (2013) Association between NQO1 C609T polymorphism and colorectal cancer risk. *Tumour Biol*., **34(6):** 4027-4032.

Welch H.G., Robertson D.J. (2016) Colorectal cancer on the decline - why screening can't explain it all. *N. Engl. J. Med*., **374(17):** 1605-1607.

Yadav U., Kumar P., Rai V. (2018) *NQO1* gene C609T polymorphism (dbSNP: rs1800566) and digestive tract cancer risk: A meta-analysis. *Nutr. Cancer*., **70(4):** 557-568.

Zhao H., Zhu F., Sun J., Meng X. (2014) Meta-analysis of the association between NQO1 Pro187Ser polymorphism and colorectal cancer in Asians. *Tumour Biol*., **35(3):** 2111-2116.

Zheng B., Wang Z., Chai R. (2014) NQO1 C609T polymorphism and colorectal cancer susceptibility: a meta-analysis. *Arch. Med. Sci*., **10(4):** 651-660.

Azərbaycan populyasiyasında kolorektal xərçəng diaqnozu qoyulan xəstələrdə *NQO1* genində C609T polimorfizminin tədqiqi

[1] Bayramov Bayram İlham oğlu, [2] Bayramov Nuru Yusif oğlu

[1] AMEA-nın Genetik Ehtiyatlar İnstitutu, Bakı, Azərbaycan
[2]Azərbaycan Tibb Universitetinin Tədris Cərrahiyyə Klinikası, Bakı, Azərbaycan

Bu tədqiqatın əsas məqsədi kolorektal xərçəng (KRX) diaqnozu qoyulan xəstələrdə *NQO1* geni C609T (rs1800566, Pro187Ser) polimorfizminin klinik əhəmiyyətini tədqiq etməkdir. Tədqiqat işinə 142 KRX diaqnozu qoyulan xəstə və 146 sağlam insan daxil edilmişdir. Qandan DNT-nin ekstraksiyası *Salting-out* üsulu ilə həyata keçirilmiş, genotiplər isə PZR-RFLP üsulu və aqaroz gel elektroforezi vasitəsilə müəyyən edilmişdir. Xəstə və nəzarət qruplarını müqayisə etdikdə heteroziqot CT (OR=1,813; 95% CI=1,097–2,995, P=0,020), dominant model (OR=1,842; 95% CI=1,137–2,983, P=0,013) və mutant T alleli (OR=1,644; 95% CI=1,096–2,465, P=0,016) ilə artmış KRX riski arasında statistik əhəmiyyətli asossasiya aşkar edildi. Həmçinin kişi xəstələri sağlam kişilərlə müqayisə etdikdə, heteroziqot CT (OR=2,219; 95% CI=1,079-4,565, P=0,029) yüksək KRX riski arasında statistik əlaqə müəyyən edildi. Bununla belə, tədqiqat qruplarının yaşı, patoloji şiş dərəcəsi və mərhələsi, siqaret və spirtli içki qəbulu müqayisə edildikdə əhəmiyyətli statistik əlaqə aşkar edilməmişdir (P>0,05). Nəticələrimiz *NQO1* C609T polimorfizminin Azərbaycan populyasiyasında kolorektal xərçəng riski ilə əlaqəli olduğunu göstərdi.

Açar sözlər: *Kolorektal xərçəng, polimorfism, PZR-RFLP, NQO1, C609T*

Исследование полиморфизма C609T гена *NQO1* у больных с диагнозом колоректальный рак в популяции азербайджана

[1] Байрамов Байрам Ильхам оглу, [2] Байрамов Нуру Юсиф оглу

[1]Институт генетических ресурсов НАН Азербайджана, Баку, Азербайджан
[2] Учебно-хирургическая клиника Азербайджанского медицинского университета, Баку, Азербайджан

Основная цель настоящего исследования состояла в том, чтобы определить клиническую значимость полиморфизма C609T гена NQO1 (rs1800566, Pro187Ser) у пациентов с колоректальным раком (КРР). В исследование были включены 142 пациента с КРР и 146 здоровых лиц. ДНК из крови выделяли методом Salting-out, а генотипы определяли на агарозном геле методом ПЦР-ПДРФ. При сравнении пациентов и контрольных групп гетерозиготная CT (OR=1,813; 95%CI=1,097-2,995, P=0,020), доминантная модель (OR=1,842; 95%CI=1,137-2,983, P=0,013) и мутантная T аллель (OR=1,644; 95% CI=1,096-2,465, P=0,016) были статистически связаны с повышенным риском КРР. При сравнении пациентов мужского пола со здоровыми мужчинами гетерозиготная CT была связана с более высоким риском КРР (OR=2,219; 95% CI=1,079-4,565, P=0,029). Однако при сравнении возраста, степени и стадии патологической опухоли, курения и употребления алкоголя в исследуемых группах достоверной статистической связи (P>0,05) выявлено не было. Результаты исследования позволили сделать вывод о том, что риск развития колоректального рака у населения Азербайджана связан с полиморфизмом C609T гена *NQO1*.

Ключевые слова: *Колоректальный рак, полиморфизм, ПЦР-ПДРФ, NQO1, C609T*

Journal of Life Sciences & Biomedicine, vol. 4(77), No 1, p. 97-105 (2022)

Studies of the engagement for serotonergic system in regulation of aggressive behavior in two behavioral models on rats

[1,2]Huseynov Shovgi Bayram, [1]*Mekhtiev Arif Alovsad

[1] *Academician Abdulla Garayev Institute of Physiology, Azerbaijan National Academy of Sciences, 78 A.M. Sharifzadeh Str., Baku AZ1100, Azerbaijan*
[2] *Jacobs School of Medicine and Biomedical Sciences, University at Buffalo SUNY, USA*
***For correspondence:** arifmekht@yahoo.com*

Received: May 02, 2022; Reviewed: May 30, 2021; Accepted: June 12, 2022

The article concerns study of the role for serotonin-modulating anticonsolidation protein (SMAP), being in linear relation with serotonin level (Mekhtiev, 2000), in regulation of aggressive behavior. The studies were carried out on aggression (induced by electroshock) and dominant (food deprivation) behavioral models, on the 5-month-old Wistar male rats. The studies were conducted in 5 series. In the 1st series of studies, undertaken on the aggression model with application of solid-phase ELISA-test, significant downregulation of SMAP ($p<0.001$) was revealed in the amygdala of the aggressive animals. In the 2nd series of studies, conducted on the aggression model, single intracerebral administration of SMAP brought to significant increase ($p<0.001$) of aggression in the animals of the experimental group, while administration of heat-inactivated SMAP to the control animals did not have any effect. In the 3rd series of studies, carried out on the dominant model, sharp downregulation of SMAP ($p<0.001$) in the amygdala of the dominant animals, though sharp upregulation of SMAP ($p<0.001$) in their platelets (corresponds to its level in the brain cortex) relatively to the intact animals were observed. In the 4th series of studies, undertaken on the dominant model, a single intracerebral administration of SMAP to the submissive animals brought to their transformation into the dominant animals ($p<0.001$), maintaining for 7-day timeframe, while inactive SMAP did not have any effect. In the 5th series of studies, conducted on the dominant model, a single intracerebral administration of rabbit polyclonal anti-SMAP antibodies to the dominant animals transformed them into the submissive animals for 1 day ($p<0.001$), while non-immune γ-globulins did not reveal any influence. On a whole, the obtained data indicate to positive regulation of aggressive behavior in the rats by SMAP and its downregulation in the amygdala of the aggressive animals in both behavioral models is, appartently, attributed to high rate of its utilization.

Keywords: *aggressive behavior, dominant behavior, Wistar male rats, serotonin-modulating anticonsolidation protein (SMAP), polyclonal anti-SMAP antibodies, indirect ELISA-test.*

INTRODUCTION

Aggressive behavior belongs to inborn behaviors. Aggressive behavior is a complex form of social behavior which advents in a context of protection or capture of resources (Nelson & Trainor, 2007). Animals demonstrate aggression to protect themselves or their progeny from the predators, to struggle for female and food and to maintain a certain hierarchic position inside the community (Popoda, 2008). Usually such behavior is characterized as undisguised behavior having goal of bringing physical damage to the other individuum (Soma et al., 2008). Some researchers define aggressive behavior as a type of agonistic behavior directed to establishment of the hierarchic dominance, getting an access to any goal or a right to any territory (Haind, 1975).

Different researchers for a long time with application of different behavioral models showed

10.5281/zenodo.7239875
Available online 30 June 2022

that aggressive behavior is triggered by neurotransmitters within the certain brain structures and the most part of such regulation is referred to neurotransmitter serotonin. The bodies of serotonergic neurons are located within the nucleus raphe in the brainstem and their axons reach each brain structure (Hornung, 2012). The most body of publications, related to the aggression studies on animal models, show that there is an inverse correlation between serotonin level in the brain structures and aggression (Carrillo et al., 2009; De Boer et al., 2009). It has been proved that upregulation of serotonergic system on account of precursors of serotonin, serotonin specific reuptake inhibitors or agonists of of receptor 5-HT_{1A} inhibit aggressive behavior (Nelson & Trainor, 2007). Furthermore, damage of nucleui raphe, being a locus of conglomeration of serotonergic neurons, brought to clearly seen downregulation of serotonin and increase of aggression in the rats: 50% of the rats, subjected to surgical damage of nuclei raphe and which had not demonstrated predatory agression prior to operation, turned to become “killers” of mice. The inverse correlation between downregulation of serotonin and increased level of aggression in the rats after damage of nuclei raphe was analyzed by administration of serotonin precursor – 5-oxytryptophan to a part of the rats after surgery, leading to suppression of aggression (Popova et al., 1978). Along with it, a number of scientists have demonstrated the existence of direct correlation between serotonin level and manifestation of aggression: in their studies 10-fold upregulation of serotonin level in the organism of the knock-out mice for tryptophan dehydrogenase synthesizing gene was accompanied with acute increase of aggression level (Shih et al., 2000).

In our earlier studies, undertaken on the conditioned shuttle-box model, the significant increase of aggression level in the rats after intracerebral administration of serotonin-modulating anticonsolidation protein (SMAP; Guseynov, Mekhtiev, 2013), being in linear relation with serotonin level (Mekhtiev, 2000), was noticed visually (biting the researcher’s hand and gnawing the iron grid floor by specimens of the experimental group). Basing on these observations, the goal of the present study was the analysis of the role of SMAP in realization of aggressive behavior in the rats on two behavioral models designed for studies of aggressive behavior.

MATERIALS AND METHODS

SMAP was purified from the cow brains through the following 2-step potocol: 1) precipitation of proteins under final 40% concentration of ammonium sulfatis; 2) gel-chromatography on the column (3 X 60 cm) of Sephadex G-150 (Mekhtiev, 2000). SMAP purification was carried out under screening control of solid-phase indirect ELISA-test with application of anti-SMAP immunoglobulins.

Anti-SMAP polyclonal immunoglobulins were produced as a result of 6-month immunization of 4 rabbits with the purified SMAP always in a mixture with equal amount of Freund’s complete adjuvant (Sigma, Germany). Blood samples were taken from the edge ear vein, serum was saved and immunoglobulins were precipitated by adding 100% ammonium sulfate solution to final concentration of 50% in the mixture.

Anti-SMAP polyclonal antibodies were purified from the solution of anti-SMAP immunoglobulins through immuno-affinity chromatography on the column (1 x 5 cm) of CNBr-Sepharose 4B with priorily immobilized SMAP. The elution procedure of the anti-SMAP antibodies, bound specifically to the affinity resin, was realized with application of chaotropic agent – 3 M potassium rodanide. In one cycle approximately 12 mg of anti-SMAP antibodies were purified from the column.

The studies were realized on the male Wistar rats having body mass 150-180 g. Behavioral studies were carried out on the aggression model, based on an electric shock stimulus, and on the dominant model, based on confronting for food.

In the 1st series the studies were carried out on the aggression model. In this model the aggression was triggered in the animals by applying the pulses of electric current to the the animals’ limbs through the iron grid floor (Rylov, Sherstnev, 1984). Electric current was changed in a step-wise manner from 0.048 to 1.5 mA. Within each group the animals were culled into pairs and each pair of the rats were put into the experimental box daily, for 5 days. The fights between the animals, initiated by electric current of the highest value (1.5 mA), were estimated as score 1, though the fights under the lowest value of electric current (0.048 mA) – as

score 42, and the fights, initiated by the intermediate values of electric current, – by the scores within 1-42 points, deployed in an inverse order to the values of applied electric current. At the end of 5th day all animals were sacrificed and amygdala was taken off from their brains, water soluble proteins were extracted and used as antigens in solid-base indirect ELISA-test on the polystyrene plates of moderate adsorption (Sigma, Germany) at a concentration of 20 μg/mL in the Tris-HCl buffer (pH 8.6). Anti-SMAP rabbit immunoglobulins were used as the first antibodies in the buffer designed for antibodies (pH7.2), and anti-rabbit goat antibodies with conjugated horseradish peroxidase were used as the second antibodies in the same buffer. Orthophenylendiamine was used as a substrate for peroxidase. The reaction was stopped 20 min later from addition of substrate solution by pouring 50 μL of 3 M NaOH into each well. The results of the reaction were registered in the photometer for the ELISA-test "Spectra Max 250" (Molecular Devices Co., USA) on the wavelength 492 nm.

In the 2nd series the studies were carried out again on the aggression model. The pairs of animals were put into the experimental box of the aggression model for 5 days, daily and thereafter 3 groups were formed: 1) intact group (n=12); 2) control group – the animals were administered with inactive SMAP (60°C on a water bath, 35 min; n=12); 2) experimental group (SMAP; n=18). The preparations were administered once, into the brain lateral ventricle of the anesthetized rats (sodium etaminali, 40 mg/kg of body mass) at a concentration 1.5 mg/ml, in a volume 10 μL, in saline, 24 h prior to the behavioral 10-day studies, in which fighting scores, according to the schedule described above, were awarded daily to each of the rat. In this series application of electric pulses was started from the lowest values, in step-wise manner increasing them to the values that induced fighting between animals.

The studies of the 3rd series were carried out on the dominant model. The experimental box, constructed from transparent organic glass, is composed of two compartments of sizes 30 X 30 X 20 cm, connected by a narrow tunnel with a small feeder, containing sweet milk, in its center (Malatynska et al., 2007). At the beginning of the studies all the rats were culled into pairs and numbered. Prior to the beginning of the studies all rats were deprived for food for 2 days, while water was given *ad libidum*. At the end of the 5th day of the daily studies the animals were sacrificed, amygdala was removed from their brains, water soluble proteins were extracted and used as antigens in the indirect ELISA-test which was conducted as in the 1st series of studies. In parallel, blood samples were taken from the rats into the sample tubes containing 5% EDTA as anticoagulation agent, centrifuged at 1,000 g for 10 min, plasma was saved into the Eppendorf sample tubes and centrifuged at 9,000 g for 20 min for platelet precipitation, which were saved and used as antigens in the indirect ELISA-test at a concentration of 20 μg/mL.

In the 4th series the studies were carried out on the dominant model. At the beginning of the studies all the rats were culled into pairs and numbered. Prior to the beginning of the studies all rats were deprived for food for 2 days. Thereafter the paired and numbered animals were culled into 3 groups: 1) intact group (n=6); 2) control group – inactive SMAP (n=6); 3) experimental group – SMAP (n=6). During the experiment the animals of the control and experimental groups were paired only with the animals from the intact group.The preparations were administered the same way as in the 2nd series of studies. The studies were conducted for 5 min, daily, during 5 days. On the 5th day the duration of staying of each animal at the feeder was recorded.

In the 5th series the studies were realized on the dominant model. The animals were culled into 3 groups: 1) intact group (n=10); 2) control group – rabbit non-immune γ-globulins (n=10); 3) experimental group – anti-SMAP polyclonal antibodies (n=10). During the experiment the animals of the control and experimental groups were paired only with the animals from the intact group. The preparations were administered the same way as in the 2nd series of studies, except for their concentration: they were used at concentration 1.8 mg/mL.

The results of studies within each series were grouped, averaged within each group and analyzed on t-Student's criterion.

RESULTS

In the 1st series of studies in the course of 5-day experiments on the aggression model gradual increase of the minimal values of electric current (corresponds to decrease of aggression scores), submited to the iron grid floor, that induced fights between specimens within pairs, was noticed.

The observed increase of the minimal values of electric current (i.e. decrease of aggression scores), required to induce fights between the rats, reflects elevation of the threshold level of onset of aggressive behavior at the end of 5-day experiment. The results of the indirect ELISA-test showed noticeable downregulation of SMAP in the amygdala of the rats of the aggressive group relatively to the intact group (0.088±0.006 vs 0.134±0.002 optic units of extinction, $p<0.001$), though in the amygdala of the submissive animals the level of SMAP (0.126±0.002 vs. 0.134±0.002 optic units, $p<0.01$) changed less prominently (Fig. 1).

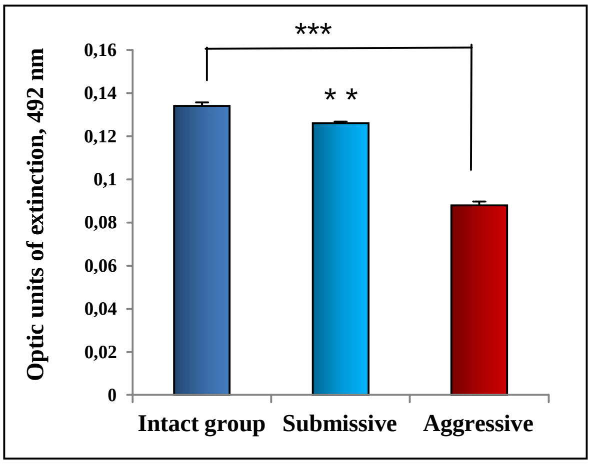


Fig. 1. Levels of SMAP in the amygdala of the rats in aggression model.**-$p<0.01$,***- $p<0.001$.

In the 2nd series of studies gradual increase of fighting scores, reflecting correspondent significant gradual decline of the threshold of aggression initiation from the score 6 prior to SMAP administration to the score 24 ($p<0.001$) on the 10th day since single intracerebral administration of SMAP was revealed (Fig. 2). At the same time no effect of inactive SMAP on the threshold of aggression initiation throughout of 10-day research was noted. These results indicate to strengthening of aggression in the rats under the effect of a single intracerebral administration of SMAP.

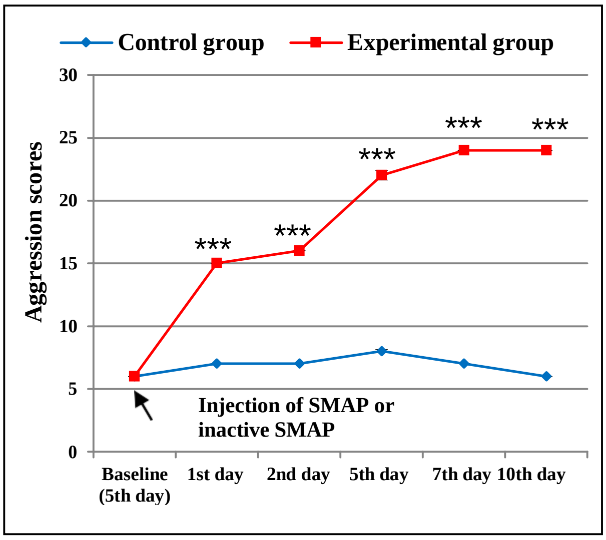


Fig. 2. Dynamics of levels of aggression after i.c. administration of SMAP. *** - $p<0.001$.

In the 3rd series of studies, carried out on the dominant behavioral model, the results of the indirect ELISA-test studies showed sharp downregulation of SMAP in the amygdala of the dominant animals relatively to its level of the intact animals (0.248±0.001 vs. 0.263±0.002 optic units, $p<0.001$; Fig. 3). In the amygdala of the submissive specimens downregulation of SMAP (0.253±0.001 vs. 0.263±0.002 optic units, $p<0.001$, Fig. 3), however, less pronounced than in the dominant animals, was as well observed. In the platelets of the dominant animals sharp upregulation of SMAP relatively to the intact animals was noted (0.253±0.002 vs. 0.237±0.003 optic units, $p<0.001$; Fig. 4), while in the platelets of the submissive animals not too prominent SMAP upregulation was observed (0.247±0.003 vs. 0.237±0.003 optic units, $p<0.05$).

In the 4th series of studies, undertaken on the dominant behavioral model, a single intracerebral administration of SMAP to the animals, defined as submissive ones in the preliminary studies, brought to their transformation into the dominant animals. In particular, if the staying time at the feeder of the submissive rats prior to SMAP administration made 109.8±7.3 sec, 24 h after SMAP administration it grew noticeably up to 180±1.5 sec ($p<0.001$; Fig. 5).

The observed effect of transformation of submissive animals into dominant ones lasted for 7 days and to the 8th day the values of staying time at the feeder declined to 141.7±1.9 sec ($p<0.001$ relatively to the values of the 1st day; Fig. 5).

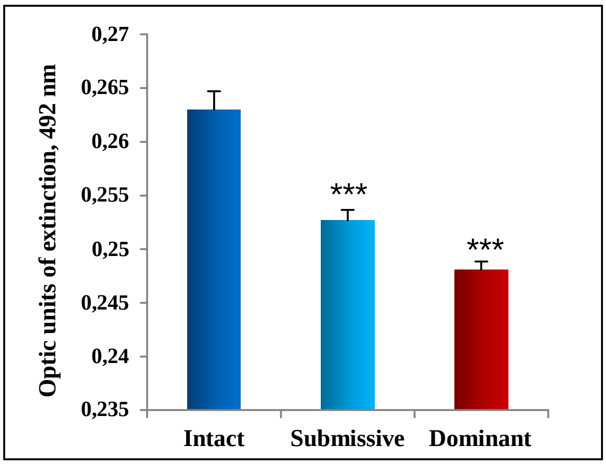


Fig. 3. Levels of SMAP in the amygdala of the rats in dominant model. *** - $p<0.001$.

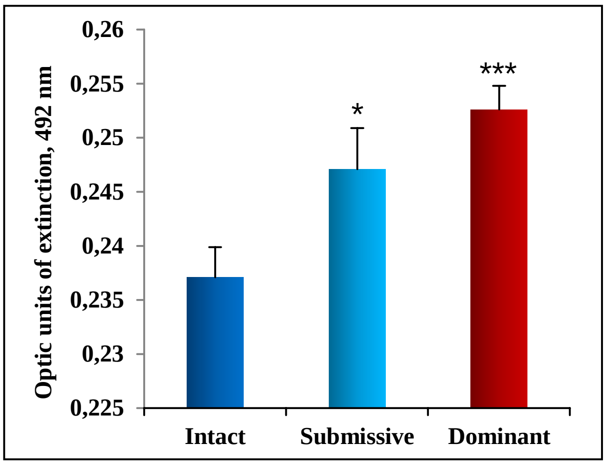


Fig. 4. Levels of SMAP in the platelets of the rats in dominant model. * - $p<0.05$; *** - $p<0.001$.

As intracerebral administration of heat-inactivated SMAP did not have strengthening effect on the original aggressive level of the submissive animals (Fig. 6), the obtained data indicate to specific effects of SMAP on animal behavior. These data support the conclusion on promoting effect of SMAP on launching aggressive behavior on the rats, noticed in the 2nd series of studies on the aggression model.

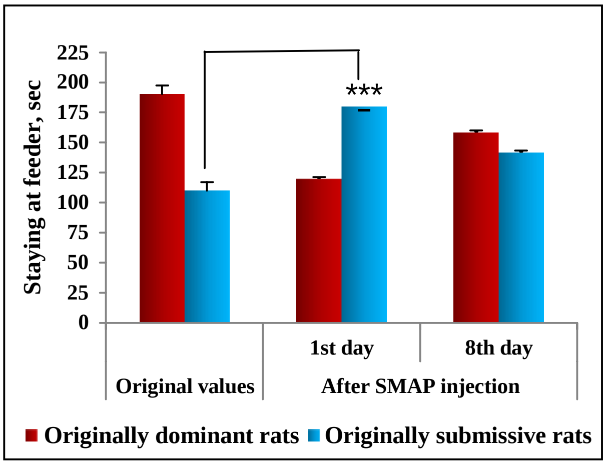


Fig. 5. Transversion of the originally submissive rats into the dominant ones after i.c. injection of SMAP in the domiunant model. *** - $p<0.001$.

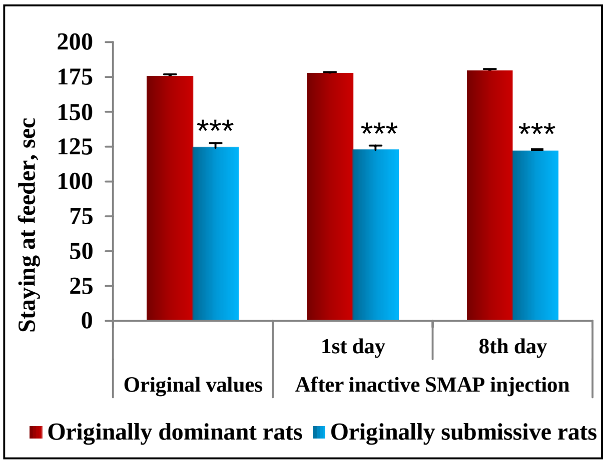


Fig. 6. Effect of i.c. injection of inactive SMAP to the originally submissive rats in the dominantg model. *** - $p<0.001$.

In the 5th series of studies on the dominant behavioral model a single intracerebral administration of anti-SMAP antibodies to the animals, defined as dominant ones in the preliminary studies, transformed them into the submissive animals. In particular, if the staying time at the feeder of the dominant rats prior to SMAP administration was 235±7.3 sec, after SMAP injection it declined drastically to 73±5.8 sec ($p<0.001$: Fig. 7). This transformation of dominant into submissive animals lasted only for 1 day, then the values of the "transformed" animals returned to the original values of staying time at the feeder, characteristic to the dominant rats (Fig. 7). At the

same time intracerebral administration of rabbit non-immune γ-globulins to the dominant animals did not change the level of their aggressiveness (not shown on the figure).

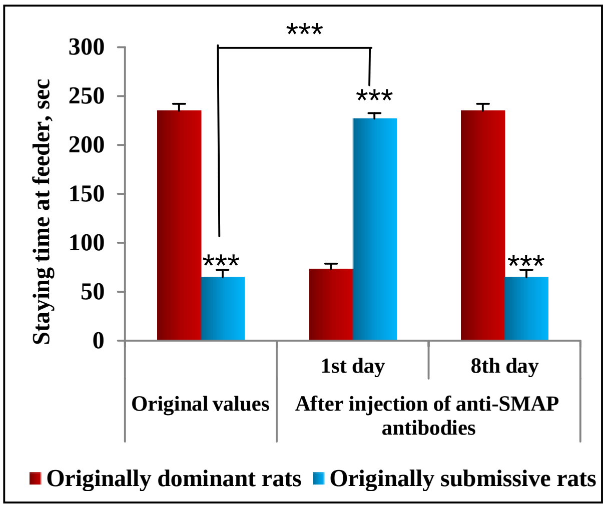


Fig. 7. Transformation of the originally dominant rats into the submissive ones after i.c. injection of anti-SMAP antibodies in the domiunant model.
*** - p<0.001.

Hence, the data show that both in the aggression and dominant models significant downregulation of SMAP was revealed in the amygdala and its upregulation – in the platelets of the aggressive animals. Intracerebral administration of SMAP to the rats brought to sharp increase of aggression level on the both models used in the study, especially prominent increase was noted in the dominance model under its administration to the submissive rats. Meanwhile, intracerebral administration of anti-SMAP antibodies to the dominant rats resulted in their transformation into submissive ones.

DISCUSSION

Although the role of amygdala as a pacemaker of aggressive emotions has already been proven by numerous studies, the brain structure, accepting plenty incoming afferent pathways from different brain structures including the amygdala and being the final brain structure responsible for regulation of animal's aggressive behavior, is the brain cortex. Serotonin turnover, its synthesizing and degrading enzymes and types of receptors in the platelets are similar to those in the brain cortex (Da Prada et al., 1988; Elliot & Kent, 1989; Collins et al., 2012). Basing on these grounds, the observed upregulation of SMAP in the platelets of the aggressive rats, apparently, reflects its upregulation in the brain cortex of these animals. In this relation, in our earlier studies, undertaken on the conditioned shuttle box model, noticeable upregulation of SMAP in the brain cortex of the control animals, which received acoustic stimulus (unconditioned stimulus) and electric shock (conditioned stimulus) in an uncombined, occasional order and, for this reason, got numerous unescapeable electric shocks, was observed (Guseinov, Mekhtiev, 2013). From this standpoint, intracerebral administration of SMAP to the submissive rats, whose original baseline levels of SMAP in the platelets (correspond to SMAP levels in their brain cortex) were lower than in the aggressive animals, should bring to their significant upregulation in the brain cortex and further – to elevation of aggression levels that was actually observed in the present study.

The effect of intracerebral administration of anti-SMAP antibodies on the aggressive annimals, "transforming" them for short-term timeframe (for 1 day) into the submissive ones, can reasonably be explained from standpoint of baseline upregulation of SMAP in the platelets (corresponds to its upregulation in the brain cortex) of the aggressive animals. In this case, the administered antibodies through blocking the SMAP activity bring to decline of the level of the active molecules of SMAP in the brain cortex of the submissive animals, which was manifested finally in lowering the aggression level to the one, characteristic to the submissive specimens.

The observed noticeable changes of SMAP level in the platelets of aggressive animals can be used in psychiatry as a biochemical marker of aggression in psychiatric disorders to prevent splashes of their manifestations or in revealing criminals, involved in terroristic affairs.

According to the available literature data, amygdala is the subcortical brain structure responsible for formation of aggression as emotion (Gouveia et al., 2019). From this standpoint, studies of the amygdala are very important for understanding the mechanisms of aggression formation. The revealed downregulation of SMAP in the amygdala of the aggressive animals on the both be-

havioral models, designed for the studies of aggressive behavior, is consistent with the results of the experimental studies on the animal models by other researchers, demonstrating inverse correlation between downregulation of serotonin and increased level of aggression (Popova, 2008).

Looking through the most body of publications demonstrating existence of the inverse correlation between downregulation of serotonin in the brain structures and increased level of aggression, it is important to perceive the underlying mechanism. The level of serotonin in the brain structures is defined by interplay of two types of enzymes: serotonin-synthesizing (tryptophan-hydroxylase type 2) and serotonin-degrading (monoamineoxidase A) enzymes. The found low level of serotonin in the amygdala of the aggressive animals might be due to either low activity of serotonin-synthesizing enzyme, or, otherwise, to upregulation of serotonin-degrading enzyme. In our studies downregulation of SMAP in the amygdala of the aggressive rats, apparently, is related to high rate of its utilization by the cells of this structure. This idea is based on the important role for SMAP in regulation of aggressive behavior, bringing to its mighty utilization, and supported by the observed increase of aggression level under intracerebral administration of SMAP both in the rats on the aggression model, and in the submissive rats on the dominant model. Conversely, decline of the aggression level as a result of antibody-mediated downregulation of SMAP molecules confirms its active engagement in positive regulation of aggressive behavior.

REFERENCES

Carrillo M., Ricci L.A., Coppersmith G.A., Melloni R.H. (2009) The effect of ıncreased serotonergic neurotransmission on aggression: A critical meta-analytical review of preclinical studies. *Psychopharmacology (Berl).*, **205(3):** 349-368; doi: 10.1007/s00213-009-1543-2.

Celada P., Puig M.V., Artigas F. (2013) Serotonin modulation of cortical neurons and networks. *Front. Integr. Neurosci*., **7:** 25; doi: 10.3389/fnint.2013.00025.

Collins1 Ch.M., Kloek J., Elliott J.M. (2013) Parallel changes in serotonin levels in brain and blood following acute administration of MDMA. *Journal of Psychopharmacology*, **27(1):** 109–112.

Da Prada M., Cesure A.M., Launay J.M., Richards J.G. (1988) Platelets as a model for neurones? *Experientia*, **44(2):** 115-126.

de Boer S.F. (2016) Untangling the neurobiology of escalated aggression in animals. Untangling the neurobiology of escalated aggression in animals. Pp. 57-72. *Paper was presented at Canine Science Forum*, 2016, Padova, Italy.

Elliott J.M., Kent A. (1989) Comparison of [125I]iodlysergic acid diethylamide binding to human fronatl cortex and platelet tissue. *J. Neurochem*., **53(1):** 191-196.

Gouveia F.V., Hamani C., Fonoff E.T., Brentani H., Alho E.J.L., Campêlo R.M., deMorais B., de Souza A. L., Rigonatti S.P., Martinez R.C.R. (2019) Amygdala and hypothalamus: Historical overview with focus on aggression. *Neurosurgery*, **85(1):** 11-30.

Guseinov Sh.B., Mekhtiev A.A. (2013) Studies of the role of serotonin-modulating anticonsolidation protein in memory formation in rats in a shuttle box. *Neuroscience and Behavioral Physiology*, **43(5):** 551-556.

Hinde R.A. (1970) Animal behaviour, a synthesis of ethology and comparative psychology. New-York, London: 856 p.

Hornung J.-P. (2012) Raphe Nuclei. **Chapter 11.** The human nervous system. 3rd ed. pp. 401-424.

Malatynska E., Pinhasov A., Crooke J.J., Smith-Swintosky V.L., Brenneman D.E. (2007) Reduction of dominant or submissive behaviors as models for antimanic or antidepressant drug testing: Technical considerations. *J. Neurosci. Methods*, **165(2):** 175-182.

Mekhtiev A.A. (2000) Revealing in the rat brain the protein possessing anticonsolidation properties. *Bulletin Exper. Biol. Med.*, **129(8):** 147-150.

Nelson R.J., Trainor B.C. (2007) Neural mechanisms of aggression. *Nat. Rev. Neurosci*., **8,(7):** 536-546; doi: 10.1038/nrn2174.

Popova N.K. (2008) From gene to aggressive behavior: The role of brain serotonin. *Neuroscience and Behavioral Physiology*, **38:** 471–475.

Popova N.K., Naumenko Y.V., Kolpakov V.G. (1978) Serotonin and behavior. Novosibirsk: Nauka, 304 p.

Rylov A.L., Sherstnev V.V. (1984) Effects of endogenic oligopeptides and brain-specific proteins on aggressive behavior in the rats. *J. Highest Nervous Activity*, **34(5):** 904-910. (in Russ)

Shih J.C., Chen K., Ridd M.J. (1999) Monoamine cxidase: From genes to behavior. *Annu. Rev. Neurosci*., **22:** 197–217.

Soma K.K., Scotti M.A.L., Newman A.E.M., Charlier T.D., Demas G.E. (2008) Novel mechanisms for neuroendocrine regulation of aggression. *Frontiers in Neuroendocrinology*, **29(4):** 476-489.

Sıçovulların iki müxtəlif davranış modelində serotoninergik sistemın aqressiya davranışının tənzimində iştirakının tədqiqi

[1,2] Hüseynov Şövqi Bayram oğlu, [1] Mehdiyev Arif Əlövsəd oğlu

[1] AMEA-nın Akademik Abdulla Qarayev adına Fiziologiya İnstitutu, Bakı, Azərbaycan
[2]SUNY Buffalo Universitetinin Cakob adına Tibb və Biotibb Elmləri Məktəbi, ABŞ

Məqalə serotonin ilə düz mütənasib əlaqədə olan (Mekhtiev, 2000) serotonin-modullu antikonsolidasiya zülalının (SMAZ) aqressiya davranışının tənzimində iştirakının tədqiqinə həsr olunmuşdur. Tədqiqatlar 5 aylıq erkək Vistar siçovulları üzərində aqressiya (elektroşok ilə yaradılan) və dominant (qidadan məhrumetmə ilə yaradılan) davranış modellərində aparılmışdır. 5 seriya tədqiqatlar həyata keçirilmişdir. 1-ci seriya tədqiqatlar aqressiya davranışı modelində və bərk-fazalı ELİSA testinin tətbiqi ilə aparılmışdır, aqressiv heyvanların amiqdalasında SMAZ-ın ($p<0.001$) miqdarının nəzərəçarpacaq dərəcədə azalması müşahidə edilmişdir. 2-ci seriya tədqiqatlar aqressiya davranışı modelində aparılmışdır, SMAZ-ın eksperimental heyvanlara birdəfəlik beyindaxili yeridilməsi aqressiya səviyyəsinin əhəmiyyətli dərəcədə ($p<0.001$) yüksəlməsinə səbəb olduğu halda yüksək temperaturun təsirindən inaktivləşdirilmiş SMAZ-ın kontrol heyvanlara yeridilməsi heç bir təsir göstərməmişdir. 3-cü seriya tədqiqatlar dominant davranış modelində aparılmışdır. SMAZ-ın miqdarının dominant heyvanların amiqdalasında intakt heyvanlara nisbətən kəskin şəkildə aşağı düşdüyü ($p<0.001$) halda, onun miqdarı əksinə olaraq dominant heyvanların trombositlərində intakt heyvanlara nisbətən ciddi dərəcədə yüksəlmişdir ($p<0.001$; beyin qabığında onun miqdarını əks etdirir). 4-cü seriya tədqiqatlar dominant davranış modelində SMAZ-ın məğlub (submissiv) heyvanlara birdəfəlik beyindaxili yeridilməsi ilə həyata keçirilmiş və nəticədə bu heyvanlar dominant heyvanlara çevrilərək ($p<0.001$) və 7 gün bu üstünlüyü saxlamışdır, lakin inaktivləşdirilmiş SMAZ-ın yeridilməsindən sonra heç bir dəyişiklik müşahidə edilməmişdir. 5-ci seriya tədqiqatlar dominant davranış modelində SMAZ-a qarşı poliklonal dovşan anticisimlərinin dominant heyvanlara birdəfəlik beyindaxili yeridilməsi ilə həyata keçirilmiş və nəticədə onlar məğlub heyvanlara çevrilərək ($p<0.001$) 1 sutka ərzində bu xüsusiyyəti saxladıqları halda, qeyri-immun γ-qlobulinlərin tətbiqi heç bir təsir göstərməmişdir. Ümumilikdə əldə edilmiş nəticələr göstərir ki, SMAZ aqressiya davranışını gücləndirir və hər iki davranış modelində aqressiv hevanların amiqdalalarında SMAZ-ın miqdarının aşağı düşməsi, güman ki, onun yüksək səvviyədə utilizasiyası ilə əlaqədardır.

Açar sözlər: *Aqressiya davranışı, dominant davranış, erkək siçovullar, serotonin-modullu antikonsolidasiya zülalı (SMAZ), SMAZ-a qarşı poliklonal anticisimlər, immuno-enzim analizi*

Изучение роли серотонинергической системы в регуляции агрессивного поведения в двух поведенческих моделях у крыс

[1,2] Гусейнов Шовги Байрам оглу, [1] Мехтиев Ариф Аловсад оглу

[1] Институт физиологии имени академика Абдуллы Гараева НАН Азербайджана, Баку, Азербайджан
[2]Школа медицинских и биомедицинских наук имени Джакоба Университета SUNY Баффало, США

Статья посвящена изучению роли серотонин-модулируемого антиконсолидационного белка (СМАБ), находящегося в прямой зависимости от уровня серотонина (Мехтиев, 2000), в регуляции агрессивного поведения. Исследования были выполнены на агрессивной (вызванной электрическим током) и доминантной (пищевая депривация) поведенческих моделях, на 5-месячных самцах линии Вистар. Исследования были выполнены в 5 сериях. В 1-й серии исследований, выполненной в модели агрессии, с помощью твёрдофазного иммуноферментного анализа было выявлено значительное снижение уровня СМАБ ($p<0.001$) в амигдале агрессивных животных. Во 2-й серии исследований, проведенной в модели агрессии, однократное внутримозговое введение СМАБ приводило к значительному увеличению уровня агрессивности ($p<0.001$) у животных экспериментальной группы, тогда как введение инактивированного нагреванием СМАБ контрольным животным не оказывали никакого влияния. В 3-й серии исследований, проведенной в доминантной модели, наблюдалось резкое снижение ($p<0.001$) уровня СМАБ в амигдале доминантных животных, тогда как в их тромбоцитах – значительное увеличение ($p<0.001$) его уровня (отражает его уровень в коре головного мозга) относительно значений интактных животных. В 4-й серии исследований, выполненной на доминантной модели, однократное внутримозговое введение СМАБ субмиссивным животным вызвало их трансформацию в доминантных ($p<0.001$), сохранявшуюся на протяжении 7-суточного интервала времени, в то время как инактивированный СМАБ не оказывал никакого влияния. В 5-й серии исследований, выполненной на доминантной модели, однократное внутримозговое введение кроличьих поликлональных антител к СМАБ доминантным животным приводило к их трансформации в субмиссивных животных на срок в 1 сут ($p<0.001$), тогда как неиммунные γ-глобулины не оказывали влияния. В целом, полученные результаты указывают на позитивную регуляцию агрессивного поведения со стороны СМАБ, а его снижение в амигдале агрессивных животных в обеих поведенческих моделях, вероятно, обусловлены высокой скоростью его утилизации.

Ключевые слова: *Агрессивное поведение, доминантное поведение, крысы-самцы, серотонин-модулируемый антиконсолидационный белок (СМАБ), поликлональные антитела к СМАБ, иммуноферментный анализ*